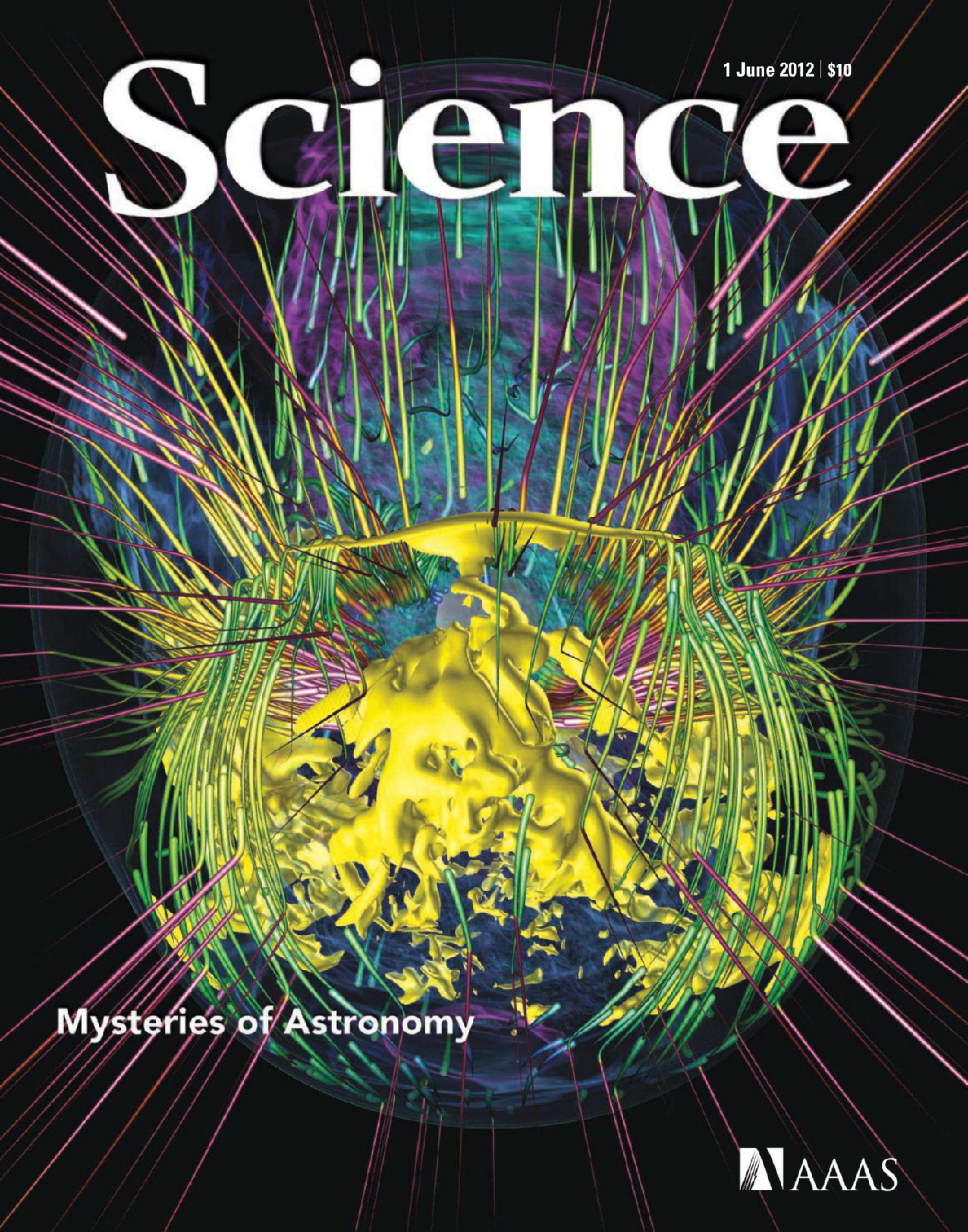


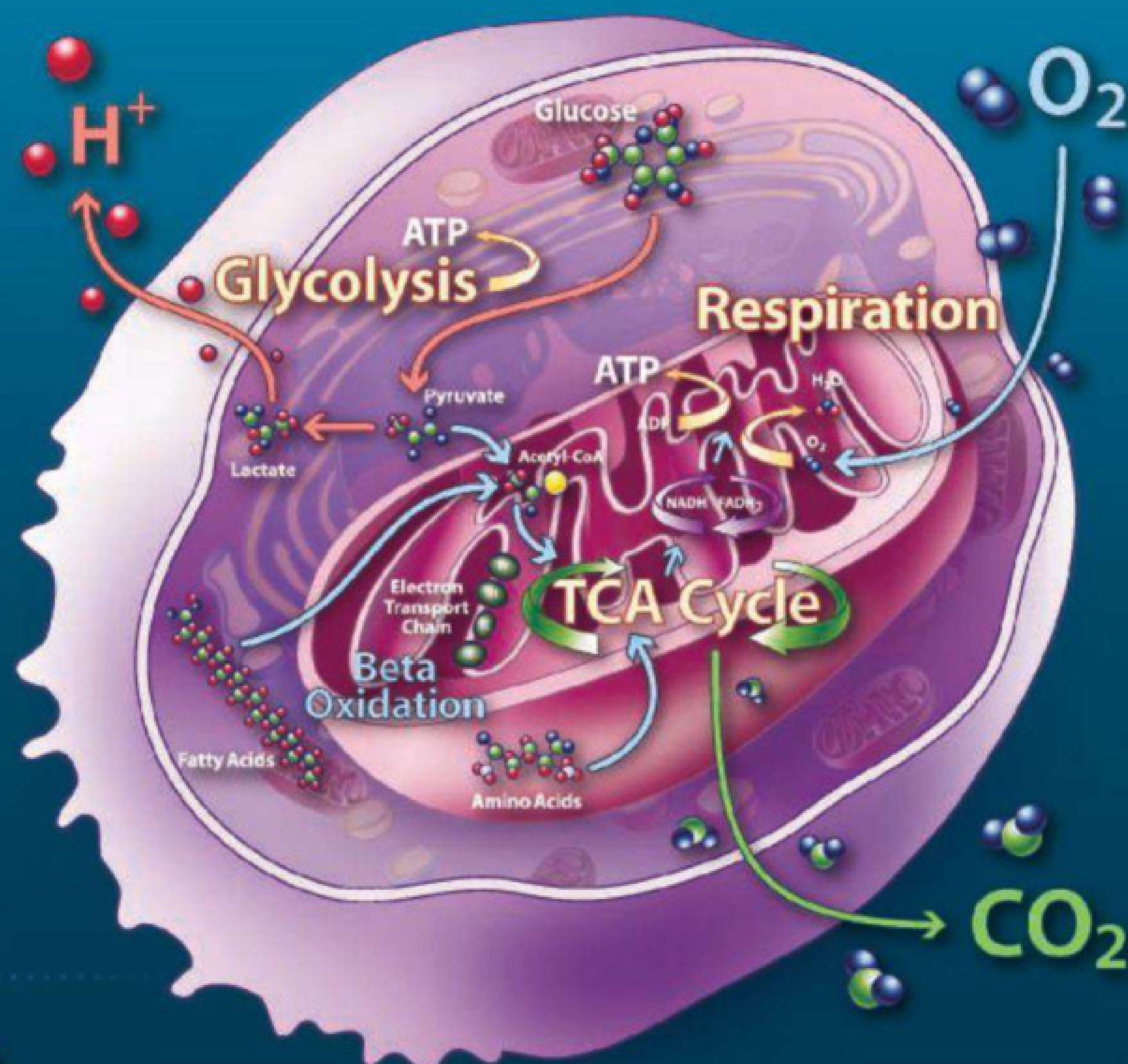


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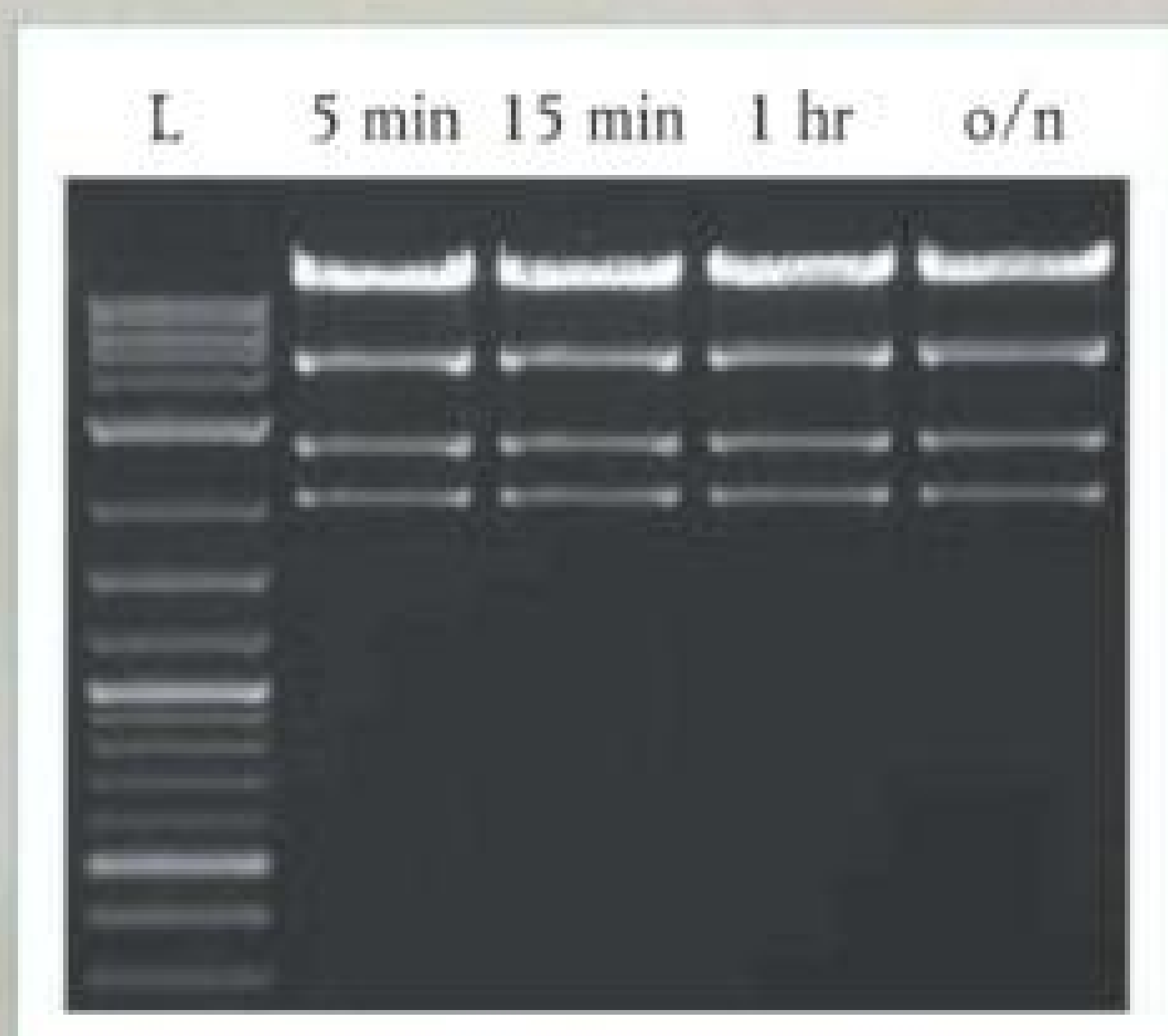
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COVER

Three-dimensional computer models such as this one help researchers explore the mechanisms behind core-collapse supernovae, the violent death of short-lived massive stars. In the image, tubes represent paths of gas falling into a supernova, deflected by an accretion shockwave (horizontal width of 600 km); colors represent different velocities. The question of how stars explode is one of the "Mysteries of Astronomy" described in a special News package beginning on page 1090.

Visualization: Hongfeng Yu and Kwan-Liu Ma, University of California-Davis and the SciDAC Institute for Ultra-Scale Visualization; Simulation: John Blondin, North Carolina State University

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BREVIA

1129 Structure of a 16-nm Cage Designed by Using Protein Oligomers

Y.-T. Lai et al.

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The elusive enzyme that modifies proteins involved in building bone and teeth has now been identified.

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L. A. O'Connell and H. A. Hofmann

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1157 Evolutionary Trade-Offs, Pareto Optimality, and the Geometry of Phenotype Space

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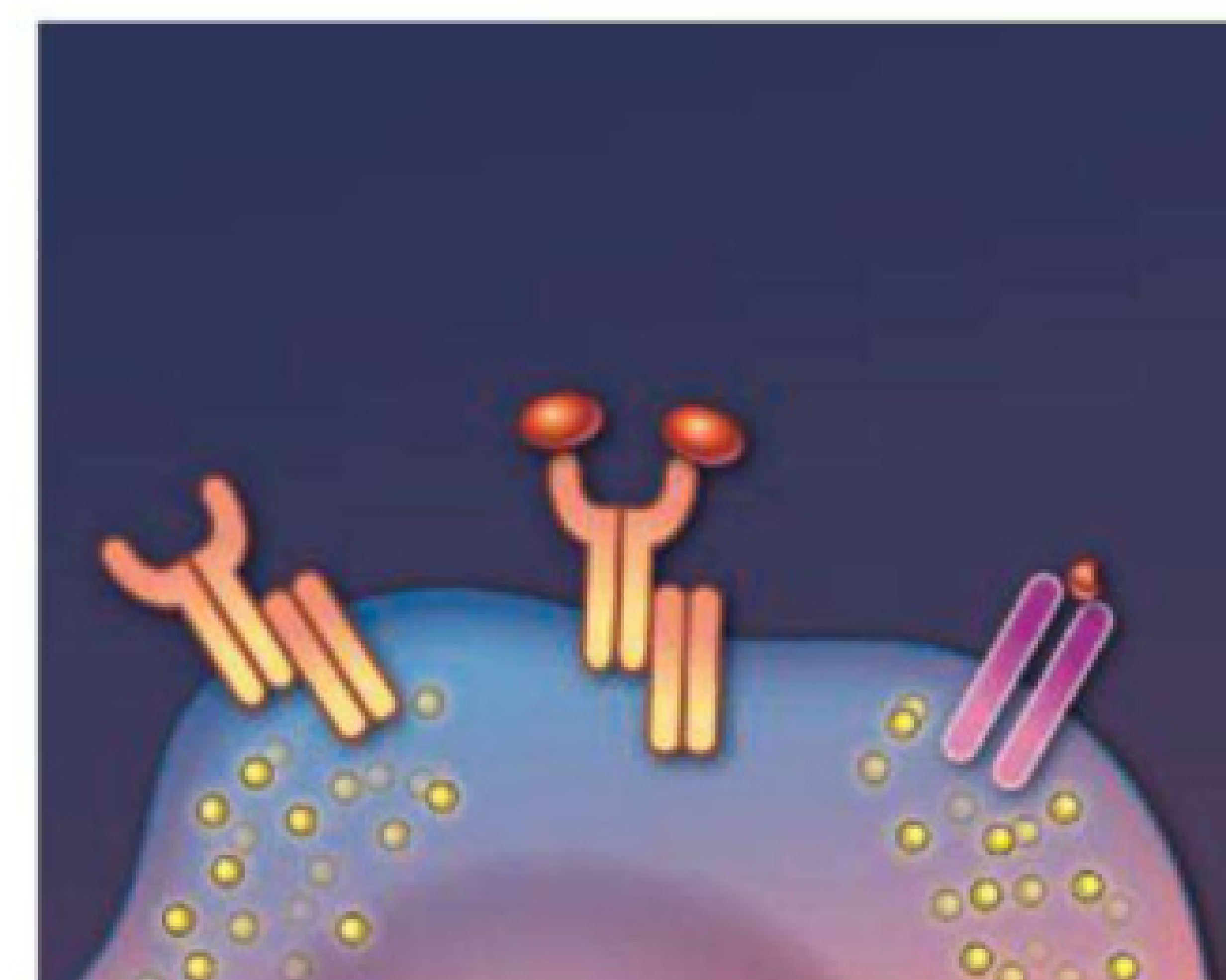
1182 Restoring Voluntary Control of Locomotion after Paralyzing Spinal Cord Injury

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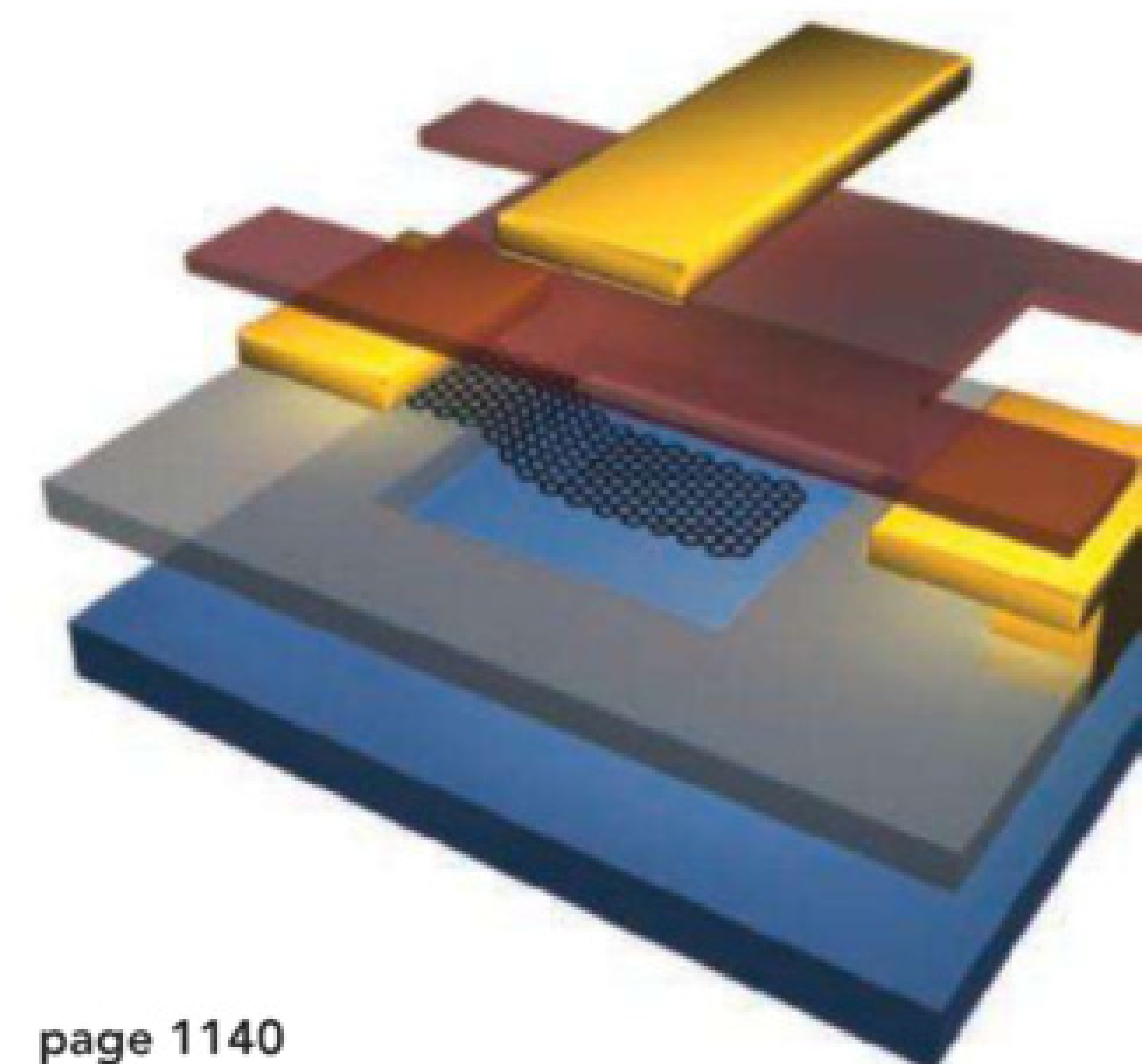
A rehabilitation program involving robotic neuroprosthetics restores previously paralyzed hindlimb function.

>> *Science Podcast*

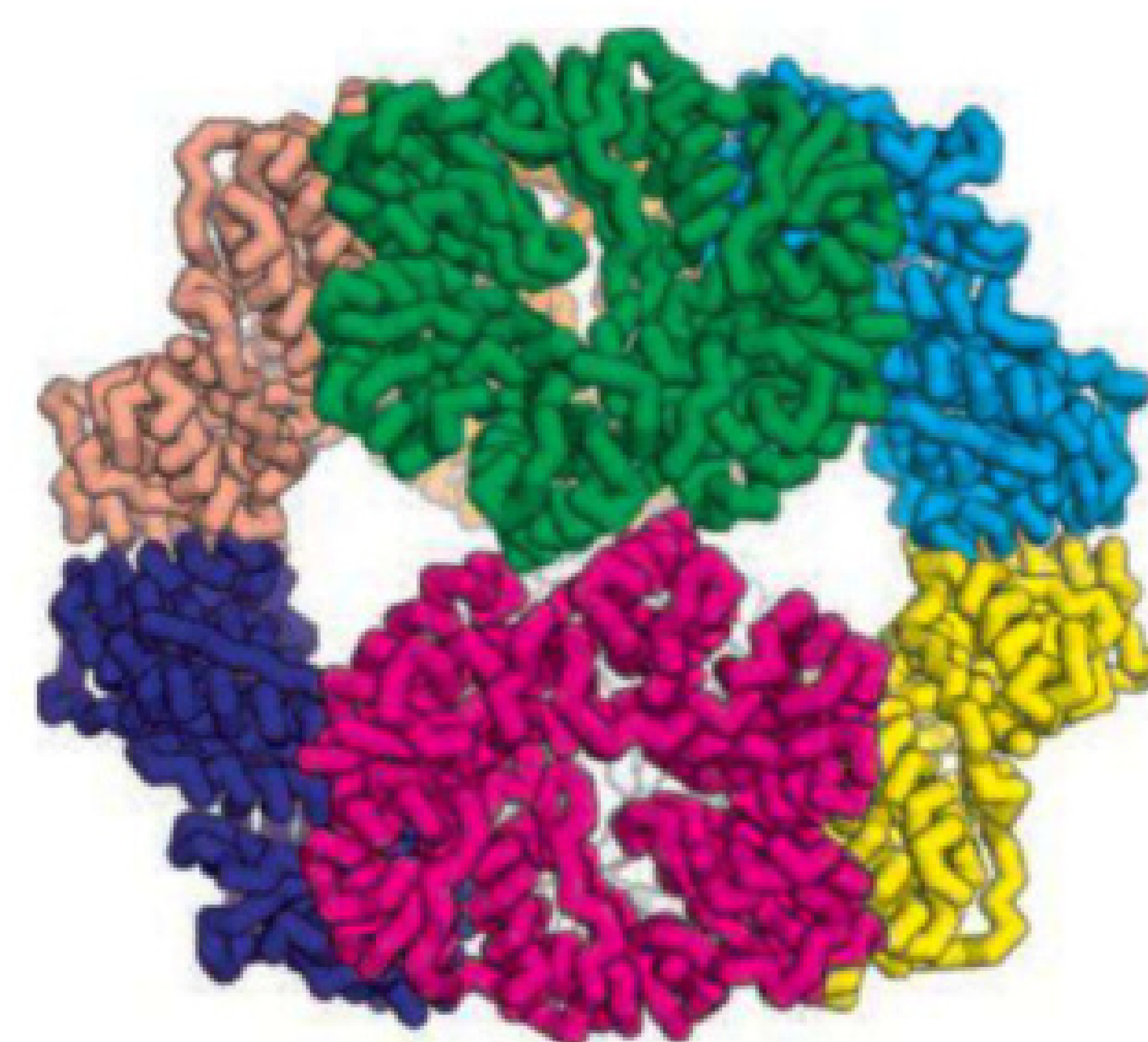
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Structural Basis of Wnt Recognition by Frizzled
C. Y. Janda et al.

The structure of the morphogen Wnt bound to its receptor provides a basis for understanding Wnt's functional pleiotropy.

10.1126/science.1222879

A Lipid Linchpin for Wnt-Fz Docking

M. Bienz and X. He

10.1126/science.1224468

Crystal Structure of the Heterodimeric CLOCK:BMAL1 Transcriptional Activator Complex

N. Huang et al.

Structure-function analyses reveal details of the interaction between two proteins that regulate daily rhythms in mammals.

10.1126/science.1222804

High-Resolution Protein Structure Determination by Serial Femtosecond Crystallography

S. Boutet et al.

A powerful x-ray laser source can probe proteins in detail using much smaller crystals than previously required.

10.1126/science.1217737

Membrane Fusion Intermediates via Directional and Full Assembly of the SNARE Complex

J. M. Hernandez et al.

During vesicle membrane fusion, straining of lipids at the edges of an extended contact zone may initiate fusion.

10.1126/science.1221976

A *Papaver somniferum* 10-Gene Cluster for Synthesis of the Anticancer Alkaloid Noscapine

T. Winzer et al.

A biosynthetic pathway inherited as a gene cluster generates a pharmaceutically useful alkaloid in poppies.

10.1126/science.1220757

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Occupy the Neolithic

Skeletons of early farmers reveal the roots of social inequality.

<http://scim.ag/Social-Inequality>

No New Neurons for Smell?

Lack of stimulation may have robbed the smell center of the human brain of new cells.

http://scim.ag/Neurons_Smell

'Asian Brown Cloud' Threatens U.S.

Continued growth in Asian pollution could warm United States.

<http://scim.ag/Asian-Pollution>

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29 May issue: <http://scim.ag/ss052912>

EDITORIAL GUIDE: Focus Issue—Signaling Architecture From Domains to Complexes

W. Wong and N. R. Gough

Structural analyses of domains, proteins, and complexes provide insight into signaling mechanisms and uncover therapeutic potential.

RESEARCH ARTICLE: Sequence-Specific Recognition of a PxLPxI/L Motif by an Ankyrin Repeat Tumbler Lock

C. Xu et al.

Phosphorylation of a motif that binds to ankyrin repeat domains switches its binding preference to 14-3-3 proteins.

REVIEW: Signal Activation and Inactivation by the G α Helical Domain—A Long-Neglected Partner in G Protein Signaling

H. G. Dohlman and J. C. Jones

Structural studies suggest that the helical domain of G protein α subunits is an active participant in G protein signaling.

REVIEW: Structural Insights into the Assembly of Large Oligomeric Signalosomes in the Toll-Like Receptor–Interleukin-1 Receptor Superfamily

R. Ferrao et al.

Structural studies show that Toll-like receptors assemble into oligomeric intracellular signaling complexes upon ligand binding.

ST NETWATCH: UCSF Chimera, PyMOL

Render structures of biomolecules in various formats, generate animations, and model binding events.

SCIENCE TRANSLATIONAL MEDICINE

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Integrating Medicine and Science

30 May issue: <http://scim.ag/stm053012>

EDITORIAL: Seeking Validation

D. Roblin

Clinical validation of new drug targets may best occur in precompetitive partnerships.

RESEARCH ARTICLE: Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

T. Forshew et al.

Sizable genomic regions were screened and low-frequency mutations were identified in circulating DNA of cancer patients using tagged-amplicon deep sequencing (TAm-Seq).

RESEARCH ARTICLE: SIV Replication in the Infected Rhesus Macaque Is Limited by the Size of the Preexisting T_H17 Cell Compartment

D. J. Hartigan-O'Connor et al.

Macaques with abundant T_H17 cells in blood and intestinal tissue before infection are resistant to SIV replication.

RESEARCH ARTICLE: Kinase-Impaired BRAF Mutations in Lung Cancer Confer Sensitivity to Dasatinib

B. Sen et al.

Induction of tumor cell senescence may explain the response of a patient with BRAF kinase-impaired lung cancer to the multikinase inhibitor dasatinib.

RESEARCH ARTICLE: A Peptide Derived from Endostatin Ameliorates Organ Fibrosis

Y. Yamaguchi et al.

FOCUS: Relief from Within—A Peptide Therapy for Fibrosis

S. P. Atamas

A naturally occurring peptide from endostatin can inhibit fibrosis in lung and skin, even when it is already established.

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V. Venkatraman

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Taken for Granted: Doing Science While Female

B. L. Benderly

A new book looks at science careers across the stages of women's lives.

http://scim.ag/TFG_ScienceWhileFemale

Career Q&A: Often Wrong, Never in Doubt

M. Fessenden

As head of the accelerator division at TRIUMF, Lia Merminga is a rare woman in the upper echelons of physics.

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Designer Hydrogels

Hydrogels, which consist of highly water swollen cross-linked polymer networks, can now be made with a range of chemistries and a combination of physical and chemical cross-links. They can also be designed to degrade gradually when exposed to chemical or biological signals and are thus finding use in a wide range of applications, including tissue engineering and drug delivery. **Seliktar** (p. 1124) reviews recent advances in tailoring hydrogels with specific properties and their applications to biology and medicine.

Quantum Leap?

Quantum computers are expected to be able to solve some of the most difficult problems in mathematics and physics. It is not known, however, whether quantum field theories (QFTs) can be simulated efficiently with a quantum computer. QFTs are used in particle and condensed matter physics and have an infinite number of degrees of freedom; discretization is necessary to simulate them digitally. **Jordan et al.** (p. 1130; see the Perspective by **Hauke et al.**) present an algorithm for the efficient simulation of a particular kind of QFT (with quartic interactions) and estimate the error caused by discretization. Even for the most difficult case of strong interactions, the run time of the algorithm was polynomial (rather than exponential) in parameters such as the number of particles, their energy, and the prescribed precision, making it much more efficient than the best classical algorithms.

Unseen Planet

The orbits of planetary bodies can be affected by gravitational interactions with other planets in the same system. If the planets can be seen to pass in front of their star (or to transit), the gravitational perturbations translate into variations in transit duration. **Nesvorný et al.** (p. 1133, published online 10 May; see the Perspective by **Murray**) inferred the presence of a far distant, previously unknown planet from the transit timing variations of a planet previously detected by the Kepler space telescope. The new planet is in a 57-day orbit and does not transit its host star. The analysis also revealed a third possible planet with a mass 1.7 times that of the Earth in a 6.8-day orbit. The radially spaced, roughly coplanar, and nearly circular orbits of the two confirmed planets mirror planetary orbits in our own solar system.

Dissecting Cooper Pairs

Angle-resolved photoemission spectroscopy (ARPES) is used in the study of the electronic structure of complex materials. Recently, time-resolved ARPES has become possible, where the state of the system is excited by a short "pump" pulse, and ARPES is performed using a second "probe" pulse applied after varying times. **Smallwood et al.** (p. 1137) used this technique to study the recombination of Cooper pairs—the fundamental charge carriers in superconductors—in a cuprate high-temperature superconductor.

Updating the Triode with Graphene

In early electronics, the triode—a vacuum device that combined a diode and an electrical grid—was used to control and amplify signals, but was replaced in most applications by solid-state silicon electronics. One characteristic of silicon-metal interfaces is that the Schottky barrier created—which acts as a diode—does not change with the work function of the metal—the Fermi level is pinned by the presence of surface states. **Yang et al.** (p. 1140, published online 17 May) now show that for a graphene-silicon interface, Fermi-level pinning can be overcome and a triode-type device with a variable barrier, a "barristor," can be made and used to create devices such as inverters.

Variation on a Theme

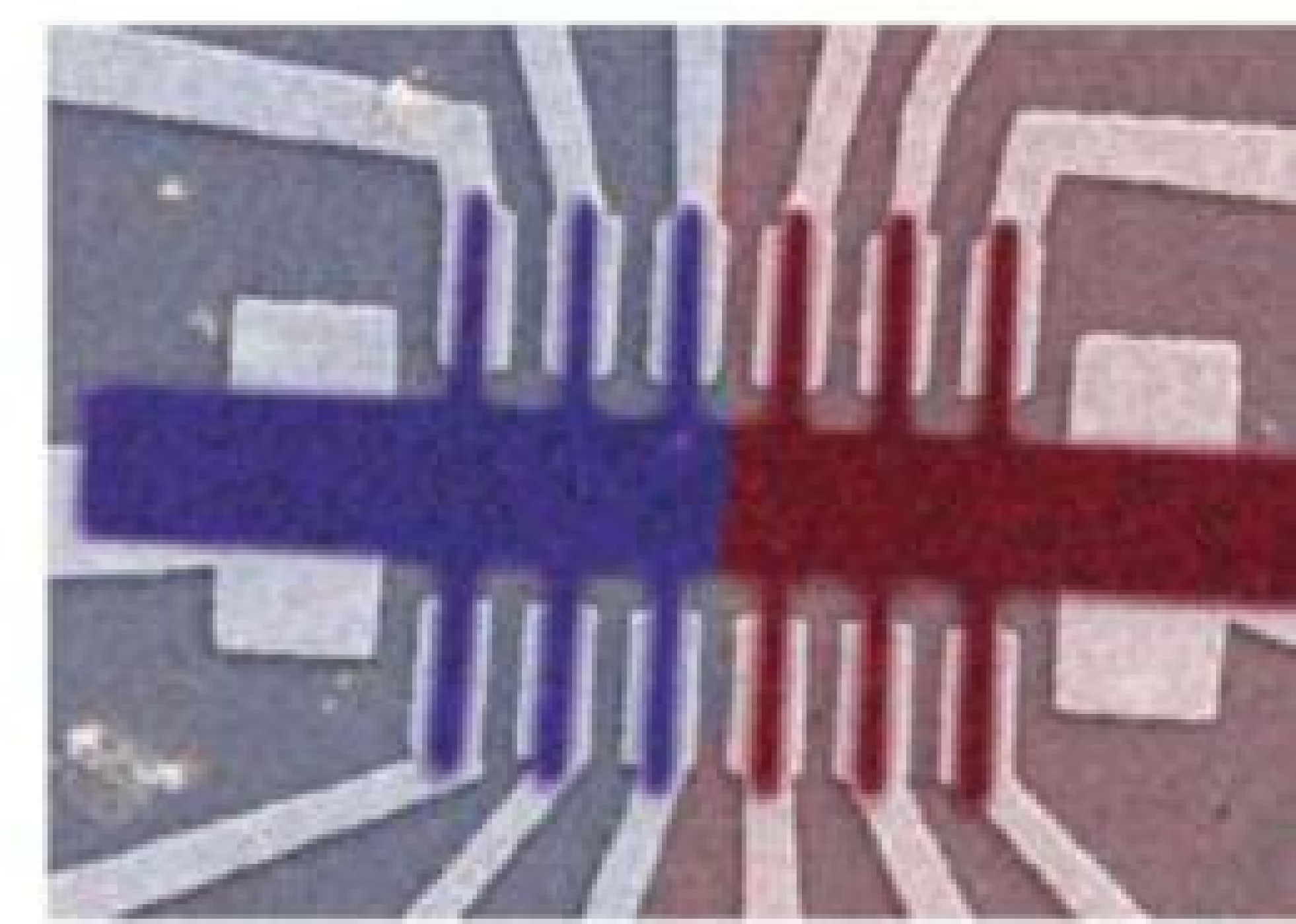
Given the incredible diversity and complexity in social behavior and ecology that exists across animal taxa, revealing the evolution of neural

mechanisms for behavior is a great challenge.

O'Connell and Hofmann (p. 1154) examined the expression profiles of several genes involved in the social behavior network and the mesolimbic reward system in 88 species across five vertebrate lineages. A remarkable level of conservation was observed in brain regions linked to social behavior and decision-making, but flexibility seems to have been maintained through variability in neuroendocrine ligand expression across the brain.

Going Up Against the Grain Boundaries

Exfoliated graphene sheets are single crystals that exhibit excellent electronic properties, but their fabrication is too slow for large-scale device fabrication. Growth methods such as chemical vapor deposition are faster, but create polycrystalline graphene sheets that contain grain boundaries that can scatter charge carriers and decrease performance. **Tsen et al.** (p. 1143) found



that the presence of overlapping domains within polycrystalline graphene samples could increase conductivity of samples by an order of magnitude, allowing them to rival exfoliated samples.

Untangling a Spectral Thicket

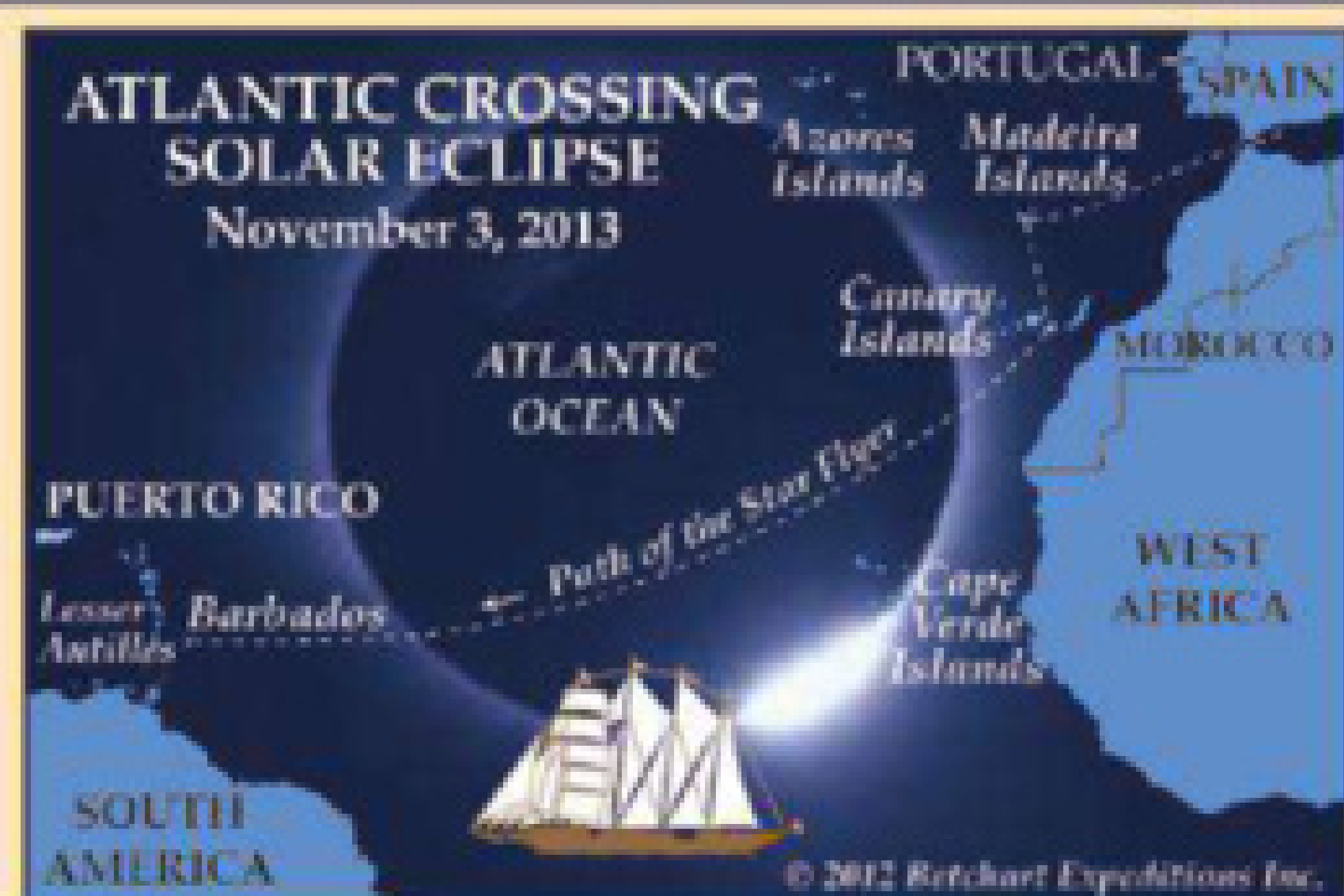
Atoms and molecules absorb characteristic frequencies of light; however, assigning the spectra of certain molecules remains a challenge. For example, the loosely bound complex of CO with H₂ produces a dense thicket of measured spectral lines that has resisted elucidation for over a decade. Now, **Jankowski et al.** (p. 1147) provide detailed theoretical calculations that match the observed pattern and thereby elucidate the vibrational and rotational states in play. The result may ultimately help to tease out the collisional dynamics of H₂ and CO in the interstellar medium.

See How They Grow

Controlled assembly and disassembly of the actin cytoskeleton is essential for processes such as cell motility, cytokinesis, and tumor metastasis. The formation of new actin filaments appears to involve the protein formin

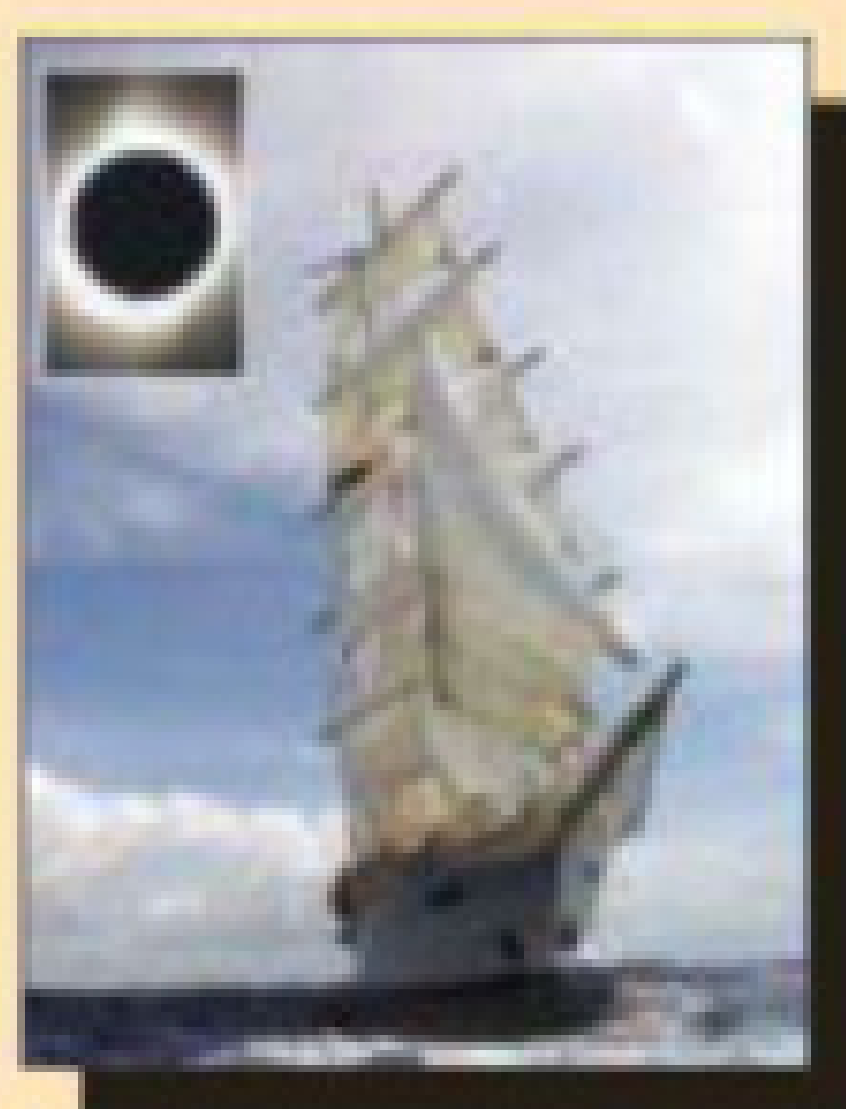
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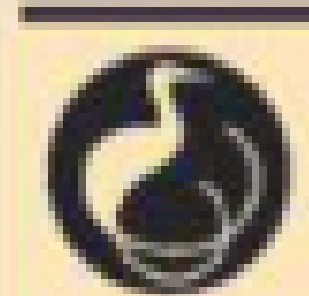


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This Week in *Science*

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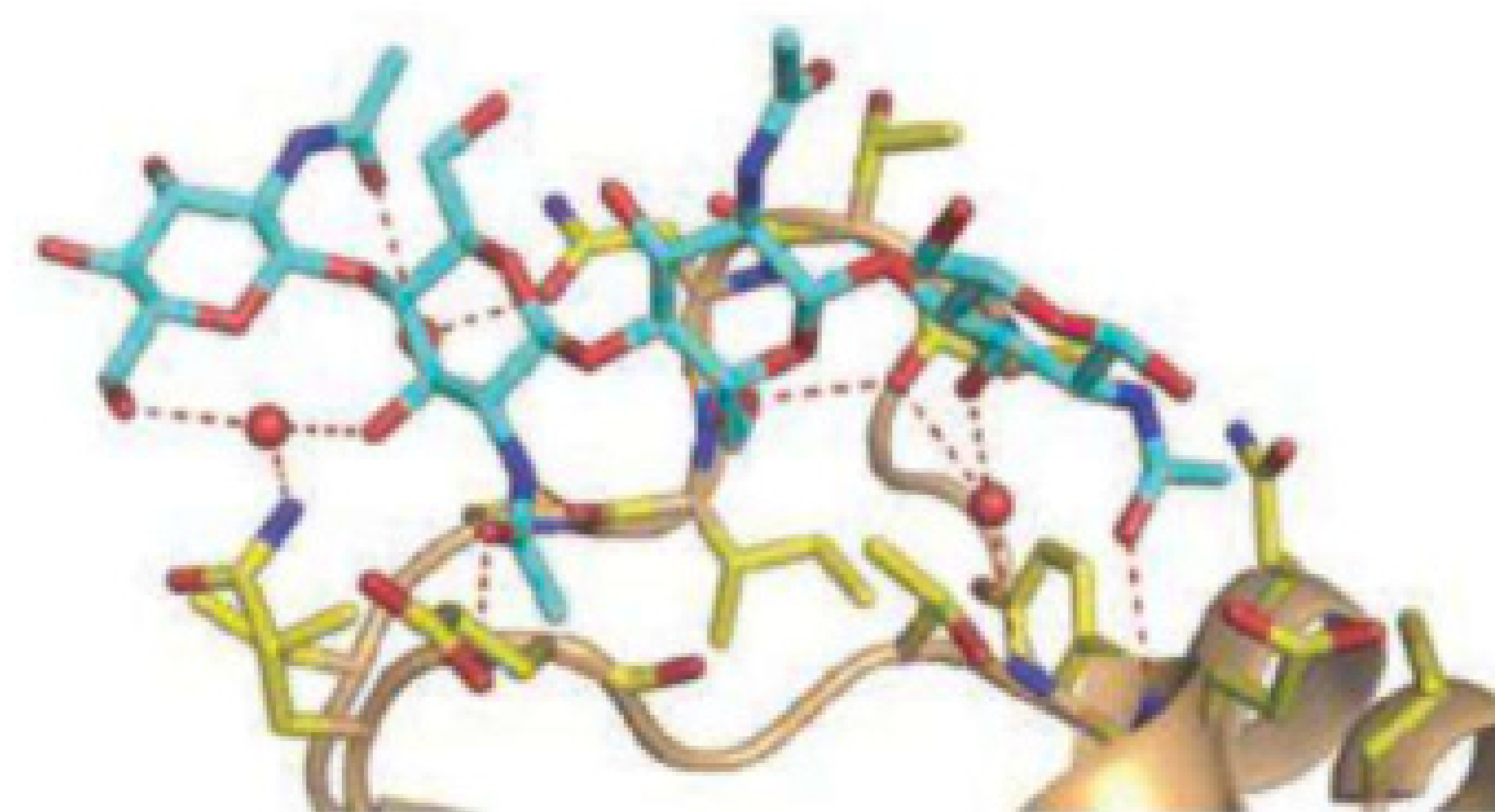
paired with another actin assembly-promoting factor. **Breitsprecher *et al.*** (p. 1164) used triple-color single-molecule fluorescence microscopy to visualize actin assembly promoted by the formin, mDia1, and the tumor-suppressor, adenomatous polyposis coli (APC). The two assembly factors interacted directly to initiate filament assembly, after which mDia1 moved with the growing barbed ends while APC remained at the site of nucleation.

Design and Build

Self-assembling biomolecules are attractive building blocks in the development of functional materials. Sophisticated DNA-based materials have been developed; however, progress in designing protein-based materials has been slower. **King *et al.*** (p. 1171) describe a general computational method in which protein building blocks are first symmetrically docked onto a target architecture, and then binding interfaces that drive self-assembly of the building blocks are designed. As a proof of principle, trimeric building blocks were used to design self-assembling 12-subunit complexes with tetrahedral symmetry and 24-subunit complexes with octahedral symmetry. **Lai *et al.*** (p. 1129) were able to build a 12-subunit tetrahedral protein cage from fused oligomeric protein domains.

Dissecting Chitin Binding

The chitin in fungal cells walls serves as a trigger to initiate plant defenses against pathogenic fungi. *Arabidopsis* detects these signals through a cell surface chitin receptor whose intracellular kinase domain initiates a signaling cascade in response to chitin that activates the plant's response to infection. **Liu *et al.*** (p. 1160) have now solved the crystal structure of the *Arabidopsis* chitin receptor AtCERK1. The results show how chitin binds to the receptor and suggest that the biological response requires dimerisation of the receptor when it binds a chitin oligomer at least seven or eight subunits long.



The Real McCoy

Some secreted proteins are phosphorylated, the most prominent example being the milk protein casein, but the enzymes that catalyze such phosphorylation have not been identified. (The proteins known as "casein kinases" are in fact cytosolic proteins and do not mediate physiological phosphorylation of casein.) **Tagliabracci *et al.*** (p. 1150, published online 10 May) searched for a human protein with the characteristics expected of a secretory protein kinase and identified Fam20C. Mutations in the gene encoding Fam20C cause defects in bone formation. Furthermore, the consensus sequence for Fam20C phosphorylation was found in several secreted proteins that function in biomineralization. Thus, Fam20C appears to be the "real" casein kinase and to function in bone physiology.

So Selective

The key elements for long-lived antibody-mediated immunity—memory B cells and plasmablasts—are generated in germinal centers, where B cells expressing high-affinity antigen receptors are selected for survival and proliferation in a process called affinity maturation. Unexpectedly, **Khalil *et al.*** (p. 1178, published online 3 May; see the Perspective by **Bannard and Cyster**) found that, in contrast to naïve B cells and B cells outside the germinal center, proximal signaling events are impaired downstream of the antigen receptor in mouse germinal center B cells.

Regaining Limb Movement

Despite many years of intensive research, there is still an urgent need for novel treatments to help patients restore motor function after spinal cord injuries. **van den Brand *et al.*** (p. 1182) produced left and right hemisections at different levels of the rat thoracic spinal cord to cause complete hind limb paralysis mimicking the situation in humans with spinal cord injury. Systemic application of pharmacological agents, combined with a multisystem rehabilitation program including a robotic postural neuroprosthesis, restored voluntary movements of both hind limbs.

CREDIT: LIU ET AL.

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Arthur Eisenkraft is the Distinguished Professor of Science Education and director of the Center of Science and Math in Context at the University of Massachusetts, Boston, MA. He has been involved in the ExploraVision competition, the Duracell/National Science Teachers Association Scholarship competition, and the NYNEX Science and Technology Awards competition, as well as in the participation of the United States in the International Physics Olympiad. E-mail: arthur.eisenkraft@umb.edu.

Competitions to Support STEM

TO PROSPER IN THE GLOBAL ECONOMY, EACH NATION MUST FOCUS ON GROWING ITS NEXT generation of scientists, engineers, and entrepreneurs. Success will require exciting many more young students about science and mathematics. When appropriately organized and directed, science and engineering competitions can provide a lifelong appreciation of, interest in, and enjoyment of science and engineering activities in much the same way that involvement in sports competitions provides a lifetime enjoyment of sporting events. In February, the second-ever White House Science Fair celebrated the student winners of a broad range of science, technology, engineering, and mathematics (STEM) competitions from across the United States. And this year will see monetary and scholarship prizes awarded to students by longstanding prestigious competitions that include the Intel Science Talent Search; the Siemens Competition in Math, Science and Technology; the Google Science Fair; the FIRST Robotics Competition; and the Toshiba/National Science Teachers Association ExploraVision competition, among others. But these competitions require financial support in much the same way that extracurricular sports and Olympic teams do. More private industries, large and small, are needed to support STEM education by sponsoring such competitions.

Many competition programs, both international and local, are supported by corporate contributions. For many students, involvement is driven by the chance of a scholarship that can range from a few hundred dollars to full support at college. In the International Science and Mathematics Olympiad competitions, top precollege students worldwide solve theoretical and experimental problems. On the local level, there are science fairs, olympics, quiz programs, and robotics competitions. Many of these competitions provide an opportunity for a student to choose and explore an original problem. Students, with guidance from teachers, scientist mentors, and other adults, work independently or in small groups on a project that they find motivating. Student participation can spill over to enhance science instruction in classrooms and provide lifelong memories of doing science; later in life, most entrants can still distinctly remember their student science fair project.

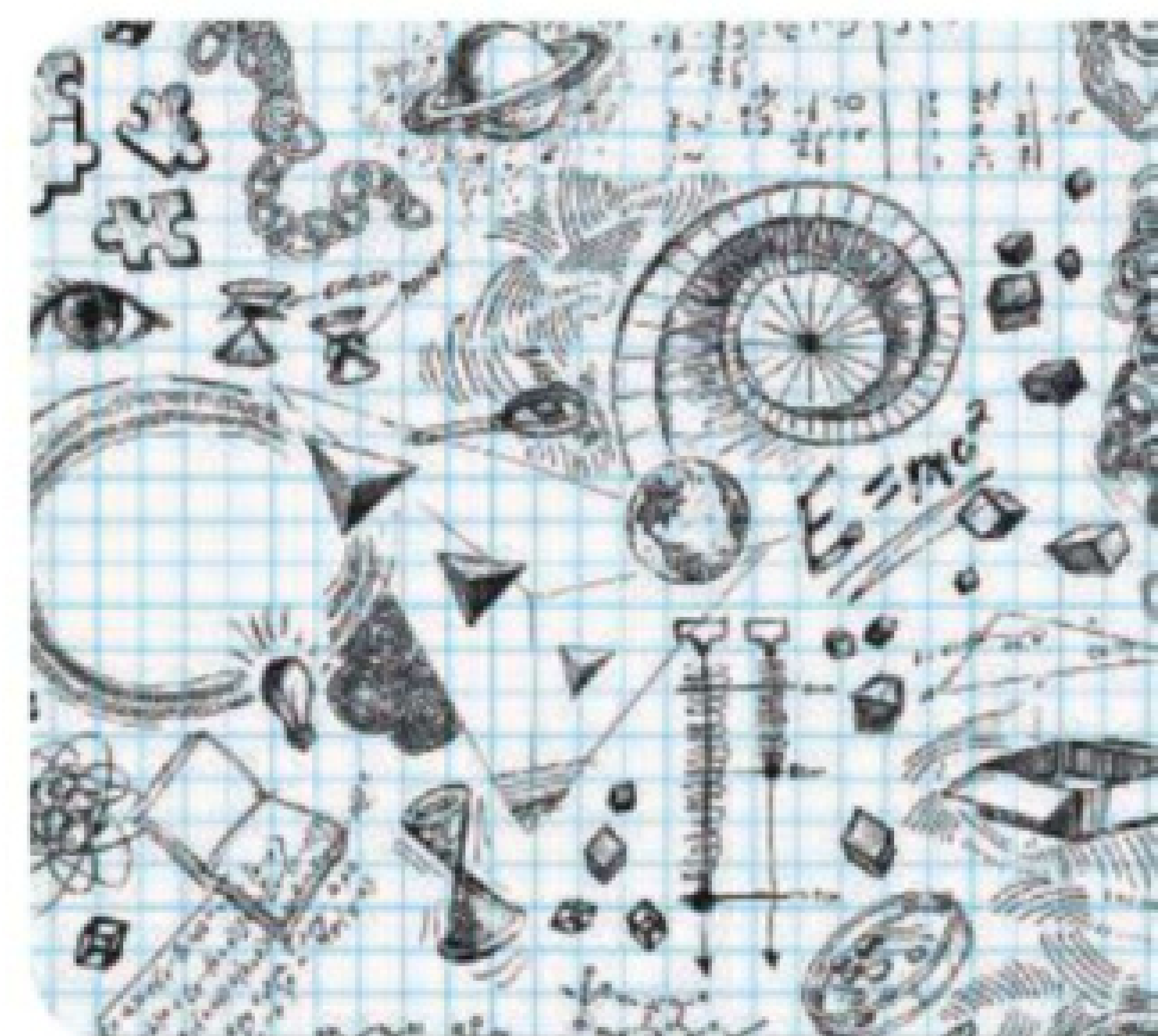
More international corporations should consider creating competitions that don't duplicate already available programs, but instead increase the range of ways in which students can be involved in STEM. It's wonderful that local companies support youth baseball and soccer teams, but they should also encourage STEM success in the schools. Most participants in major competitions started with simpler competition projects; thus, this is an approach that keeps students engaged and motivated over the years, building both interest and skills. Unfortunately, many industries lack the staying power to remain committed to supporting STEM education. Too often, marketing departments of large corporations must decide between funding an educational initiative or putting their company's logo on a banner at a sporting event. STEM education often loses that competition.

Despite 8% unemployment today, the United States cannot fill 600,000 jobs that require analytical and technical skills.* Business needs to think in the long term: The success of longstanding and widely recognized competitions, such as Intel and Toshiba, should serve as models for other large corporations to explore. Teachers and scientists can work with local industry to start a science fair or an invention competition. Producing future graduates with STEM skills must become a universal goal, and meeting it will be critical for the economic and societal well-being of each and every nation.

— Arthur Eisenkraft

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*www.themanufacturinginstitute.org/~media/A07730B2A798437D98501E798C2E13AA.ashx.



EDITED BY KRISTEN MUELLER AND JAKE YESTON

ECOLOGY

The Power of Pollination

In the context of contemporary climate change, there has been a resurgence of interest in understanding the factors and processes that influence the geographical distribution of species. Most research on this question, not surprisingly, has focused on physical factors—particularly temperature and precipitation. The influence of biotic factors and species interactions such as mutualism on distributions has received less attention.

Moeller *et al.* examined how the strength of a mutualistic interaction varied across a species' range. The herbaceous plant *Clarkia xantiana*, which is endemic to California, is pollinated by a suite of insect species. A study over 4 years showed a consistent decline in the abundance of pollinators from the center to the edge of the plant's geographic range, and experimental manipulations of the plants confirmed that reproduction at the range limits was limited by pollen availability and was not compensated for by self-pollination. Although the ultimate cause may be climatic (the pollinators' abundance itself being influenced by precipitation), this study provides new insight into the multiple factors that control biogeographic patterns. — AMS

Ecology **93**, 1036 (2012).



EDUCATION

Automate to Educate

Science education reforms face a daunting challenge: How do we assess skills that cannot be easily automated or digitized? Nehm *et al.*, examined the capacity of the software package Summarization Integrated Development Environment (SIDE) to automatically analyze and score written explanations of evolutionary change. Using an online response system, 2260 writing samples from 565 undergraduates with varying levels of evolution knowledge were collected and graded by two human raters. Human-scored responses were used to train SIDE software, and human- and software-scored samples were compared. SIDE performance was found to be most effective when used at the individual item level; that is, SIDE was most effective when it was trained with the same type of items that it subsequently scored. SIDE was found to be advantageous over current commercial text analysis programs, because it required less time and financial investment. Because SIDE can be used in areas outside of biology, it has potential to become an important tool for educators as science education moves to include more authentic problem-solving tasks. — MM

J. Sci. Educ. Technol. **21**, 183 (2012).

PHYSIOLOGY

Working on Borrowed Time

Many of us are sleeping less than we used to because of the demands of work and the enticements of the Internet, television, and digital

social networking. It is also true that we are increasingly sleeping outside of the times normally dictated by our internal circadian clocks (our "chronotype"). This difference between circadian and social clocks has been termed "social jet lag."

Roenneberg *et al.* have analyzed data from the Munich ChronoType Questionnaire (MCTQ), which assesses sleep behavior on work and free days. They calculated that one-third of the 65,000 European participants in the MCTQ suffered from at least 2 hours of social jet lag, with teenagers suffering the largest deficiencies. Reduced amounts of sleep are known to be correlated with increased body mass index (BMI) and obesity—the results showed that social jet lag is an equally important predictor



of BMI. Furthermore, the average chronotype has shifted later into the night over the past decade, exacerbating social jet lag. This change in chronotype has probably been driven by a weakening of the external cues that normally entrain our circadian clocks—increasing

numbers of people living and working in cities being exposed to less light during the day and more light during the night, and spending less time outdoors. People who regularly sleep outside of their circadian window can show an imbalance in glucose metabolism normally associated with type 2 diabetes. — GR

Curr Biol. **22**, 10.1016/j.cub.2012.03.038 (2012).

APPLIED PHYSICS

Graphene's Plasmon Prospects

The length scales pertaining in optics and nanoelectronic circuitry differ by orders of magnitude. Combining the two offers the potential of exploiting the ultimate in transmission speed (the speed of light) with the tens of nanometers scale available to state-of-the-art chip fabrication facilities. Plasmons are light-induced collective electronic excitations usually found confined to the near-surface region of metals, and it is this deep subwavelength confinement that is thought to offer the potential for providing that bridging capability. However, plasmons tend to suffer from short propagation lengths, making plasmon circuitry design a challenge. Thongrattanasiri *et al.* explore the possibility of using graphene as a platform to support the plasmonic excitations. Their numerical work shows that the longer lifetime of the plasmonic excitations in graphene, taken together with the ability to alter the transport properties via patterned electrostatic gating of the material, should provide a promising route toward realizing a viable plasmon circuit technology. — ISO

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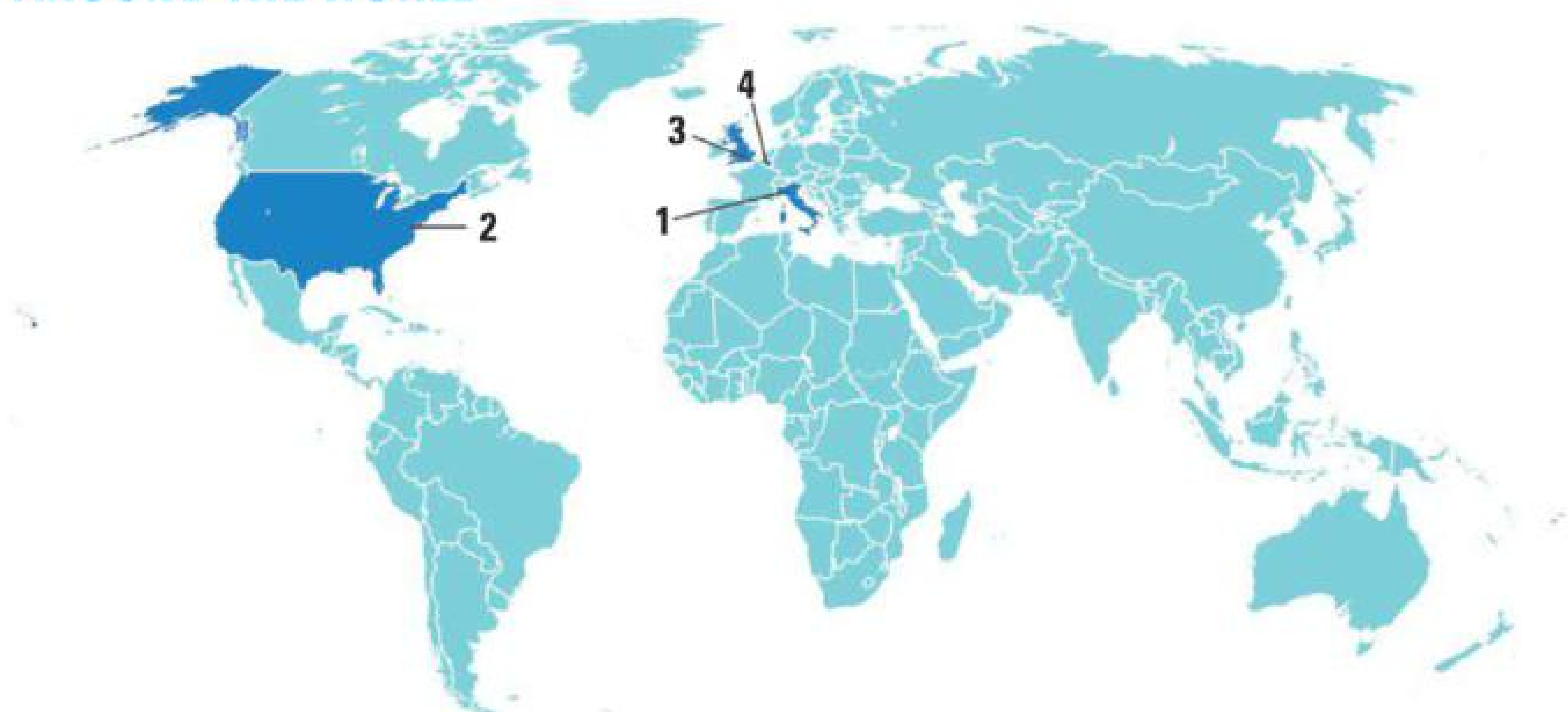
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Richard Schweder, Univ. of Chicago
Ed Wasserman, DuPont
Lewis Wolpert, Univ. College London

AROUND THE WORLD



Parma, Italy 1

French GM Concerns Dismissed—Again

France's latest attempt to keep genetically modified (GM) crops from its fields was rebuked by a scientific panel at the European Food Safety Authority (EFSA) on 21 May. EFSA dismissed France's argument that a GM maize variety produced by Monsanto called MON810 might be harmful to the environment or human health.

The European Commission approved the cultivation of MON810 in 1998, but the French government, faced with strong public opposition to GM crops, banned MON810 in 2008. EFSA rejected France's measure later that year.

France again asked the European Commission in February for permission to ban



MON810, suggesting that Cry1Ab, a protein produced by MON810 to ward off maize stalk borers (shown), could hurt non-target species such as bees and butterflies, and that it could linger in the soil.

But the EFSA Panel on Genetically Modified Organisms said that it "could not identify any new science-based evidence indicating that maize MON 810 cultivation in the E.U. poses a significant and imminent risk to the human and animal health or the environment." <http://scim.ag/EFSAdismiss>

Washington, D.C. 2

Senate Committee Wants to Sink Military's Biofuels Program

The Senate Armed Services Committee narrowly voted on 24 May to ban the Department of Defense (DOD) from paying more for alternative fuels than for petroleum-based supplies and from building a biofuel refinery unless explicitly authorized to do so. The House of Representatives agreed to similar bans last month (*Science*, 25 May 2012, p. 971). Critics say the measures undermine efforts to diversify fuel sources and make the country less dependent on foreign oil. The measure is expected to be taken up by the full Senate in June.

To reduce the impact of oil shortages and price spikes on its budget, DOD has pushed a program of investing in alternative energy sources since the Bush Administration. The Navy and Air Force set goals of using advanced biofuels, which can be blended with petroleum and burned in conventional engines, for 50% of their fuel use by the end of this decade. Military officials insist their investments in alternative energy are intended to improve energy security and drive down biofuel costs. But congressional Republicans have accused the Obama Administration of using the military to support its green agenda.

<http://scim.ag/DODbiofuels>

Harpenden, U.K. 3

Test Crop Survives Protest

An experiment to test a strain of genetically modified wheat can continue after police prevented activists from destroying the crop. A group called Take the Flour Back had announced their intention to "decontaminate" the field trial at a day of protest on



27 May at the Rothamsted Research agricultural research station in Harpenden, U.K. Police lines blocked several hundred protesters from entering the test site, and after enjoying "a GM-free picnic" and listening to speeches, the protesters dispersed peacefully.

Online, however, Rothamsted was subject to another, less peaceful, attack that took its Web site down for several hours shortly after the protest ended.

Brussels 4

Horizon 2020: Battlefield For Open Access

Last week, the European Commission's research director, Robert-Jan Smits, said that open access "will be the norm" for studies funded through Europe's £80 billion Horizon 2020 research program. But what that will mean is unclear. Open access typically involves making research papers freely available, but the commission hasn't said whether it will require that papers be placed in a public repository immediately or within months or a year of publication. Horizon 2020, the successor of the current Framework Programme 7 (FP7), will start in 2014. Open access was partly mandatory in FP7, but the European Commission currently has no power to enforce compliance from scientists. <http://scim.ag/2020access>

NOTED

>Tweeters, prepare your hashtags: The Innovative Medicines Initiative, a joint venture between the European Union and Europe's pharmaceutical industry, last week launched a new €220 million collaboration of companies and public partners to combat antimicrobial resistance—and they're calling it **NewDrugs4BadBugs**. The name might not bug the European Union for long, though: Like an adapting microorganism, it is starting to hide behind its own acronym: ND4BB.

THEY SAID IT

"We called it the banana standard. We thought this was a great idea, to show people that any radiation doses experienced by Americans from Fukushima would be small compared to eating a banana. But apparently some people in the banana industry took offense."

—U.S. presidential science adviser John Holdren describing a risk-communication strategy (which was never implemented) after the March 2011 Fukushima nuclear disaster, based on the natural radiation from potassium-40 in a banana.

NEWSMAKERS

Radioactive Waste Expert Nominated to Lead NRC

Allison Macfarlane, an academic geologist and nuclear waste expert, is in line to be the next head of the U.S. Nuclear Regulatory Commission (NRC). President Barack Obama's choice last week of Macfarlane, a professor at George Mason University (GMU) in Fairfax, Virginia, is drawing positive reviews from key members of Congress and both supporters and critics of nuclear power.

Macfarlane is a veteran of nuclear power policy debates. She earned a 1992 doctorate in geology from the Massachusetts Institute of Technology in Cambridge, and joined GMU in 2006 after a succession of academic posts at top-tier institutions. In 2010 she was named to the presidential Blue Ribbon Commission on America's Nuclear Future.

If confirmed by the Senate, Macfarlane would replace Gregory Jaczko, a physicist and former aide to Senator Harry Reid (D-NV), the Senate's Majority Leader. Jaczko announced on 21 May that he would step down following controversy over his management style and policy positions. Reid said he hopes the Senate will vote on Macfarlane's nomination by the end of June. <http://scim.ag/Macfarlane>



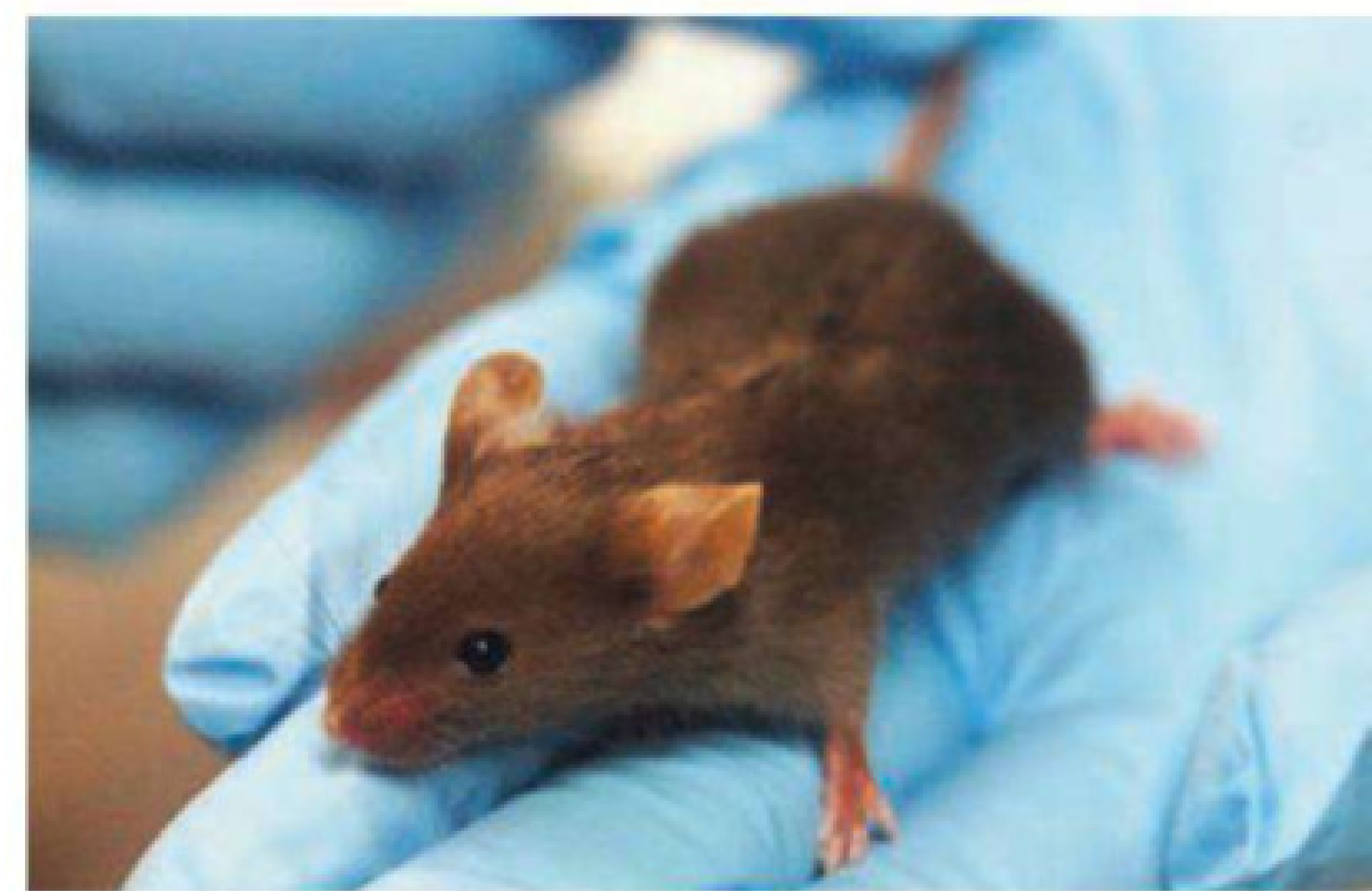
Macfarlane

FINDINGS

Jet Lag Disrupts Pregnancies in Mice

What happens when you give a mouse jet lag? If she's pregnant, the results could be disastrous. Researchers placed laboratory mice that had recently copulated on a regimen of artificial daylight. Then they shifted the rodents' exposure to this light forward by 6 hours every 5 to 6 days—the equivalent of flying from Chicago to London. Four time shifts later, only 22% of the mice gave birth compared with 90% in a control group. Mothers may have reabsorbed their pregnancies or fertilized eggs might never have implanted, the team reported online 23 May in *PLoS ONE*.

The result fits with previous studies which found that mice with mutations in genes that regulate their circadian rhythms have irregular estrous cycles and more pregnancy failures. Shift workers and flight attendants, whose own body clocks are disrupted, also report increased miscarriages and menstrual changes. But there may be hope: When mice were clock-shifted back-



ward rather than forward, the effect was less severe. So even if circadian changes lead to fertility problems in women, careful scheduling might limit the turbulence.

No New Neurons in Adult Olfactory Area

For decades, scientific wisdom held that the birth of new neurons, called neurogenesis, wasn't possible in the adult brain. But studies since the late 1990s have shown that neurogenesis occurs in humans in a memory nexus known as the hippocampus. Now, a surprising study shows that >>



Hitchhikers From the Deep

Are deep-sea submersibles bringing back creatures from the ocean depths? Scientists have long argued that immense pressure differences would kill any hangers-on. But, as reported online 24 May in *Conservation Biology*, some organisms from sea-floor vent systems may survive long enough to travel to another undersea spot.

In September 2004, the submersible *Alvin* slurped up sediments from the sea floor at a site more than 2200 meters deep off the coast of Washington. But some of the creatures in the sediments—especially limpets (shown), a type of marine snail—turned out to be stowaways that had hitched a 635-kilometer ride with *Alvin* from a hydrothermal vent the researchers had visited 2 days earlier. The ratios of carbon and nitrogen in the creatures' tissues and shells, and the ratio of males to females in the sample—characteristics that vary among hydrothermal vent populations—gave the stowaways away.

Random Sample



Bivalve Digging Inspires 'RoboClams'

The speedy digging strategy of a humble clam may help underwater robots stay parked over the sea floor.

As any clam-digger knows, Atlantic razor clams (*Ensis directus*) are quite spry, despite being legless. They can burrow 70 centimeters deep into sediment at up to a centimeter per second, although their muscles suggest they should be much slower.

So what keeps them from slowing down as they tunnel deeper? The trick, discovered mechanical engineer Amos G. Winter V of the Massachusetts Institute of Technology in Cambridge, is that the clams surround themselves with a pocket of quicksand. Winter and his team placed razor clams inside a tank filled with small glass beads. The clams first wriggled part of their shells into the "sediment" with a fleshy organ called a foot. Then, they contracted their shells so that the surrounding beads started to cave in. By pulling their shells in even closer, the clams drew surrounding water into the spaces that opened up between the beads. The resulting water-bead mixture reduced the resistance clams encountered while digging, the researchers reported online 23 May in *The Journal of Experimental Biology*.

Based on the animals' technique, Winter successfully tested a prototype burrowing robot, RoboClam, and is now busy building small robots that mimic the clam's motions for inclusion on autonomous underwater vehicles. The goal is to use them as anchors that dig themselves in and out of the sea floor, eliminating the need for battery-draining motors while still keeping the vehicle stationary in the water.

>>FINDINGS

the same may not be true for the olfactory bulb—a known hot spot of neurogenesis in other animals.

Jonas Frisén and colleagues at the Karolinska Institute in Stockholm used radio-carbon dating to test human brain tissue taken in autopsies. The technique takes advantage of nuclear testing during the 1950s and early

1960s, which released massive amounts of the isotope carbon-14 (^{14}C) into the atmosphere. Because ^{14}C is taken up into the cells of plants and animals, and thus the humans that eat them, the isotope acts as a date stamp for the formation of new cells. In the 23 May issue of *Neuron*, the researchers report that olfactory neurons are all the same age—the age of the individual they were taken from.

Tippy-Top Target for Next Mars Rover

When NASA's rover Curiosity arrives at Mars on 6 August, its prime target will be Mount Sharp, the 5-kilometer-high mound

BY THE NUMBERS

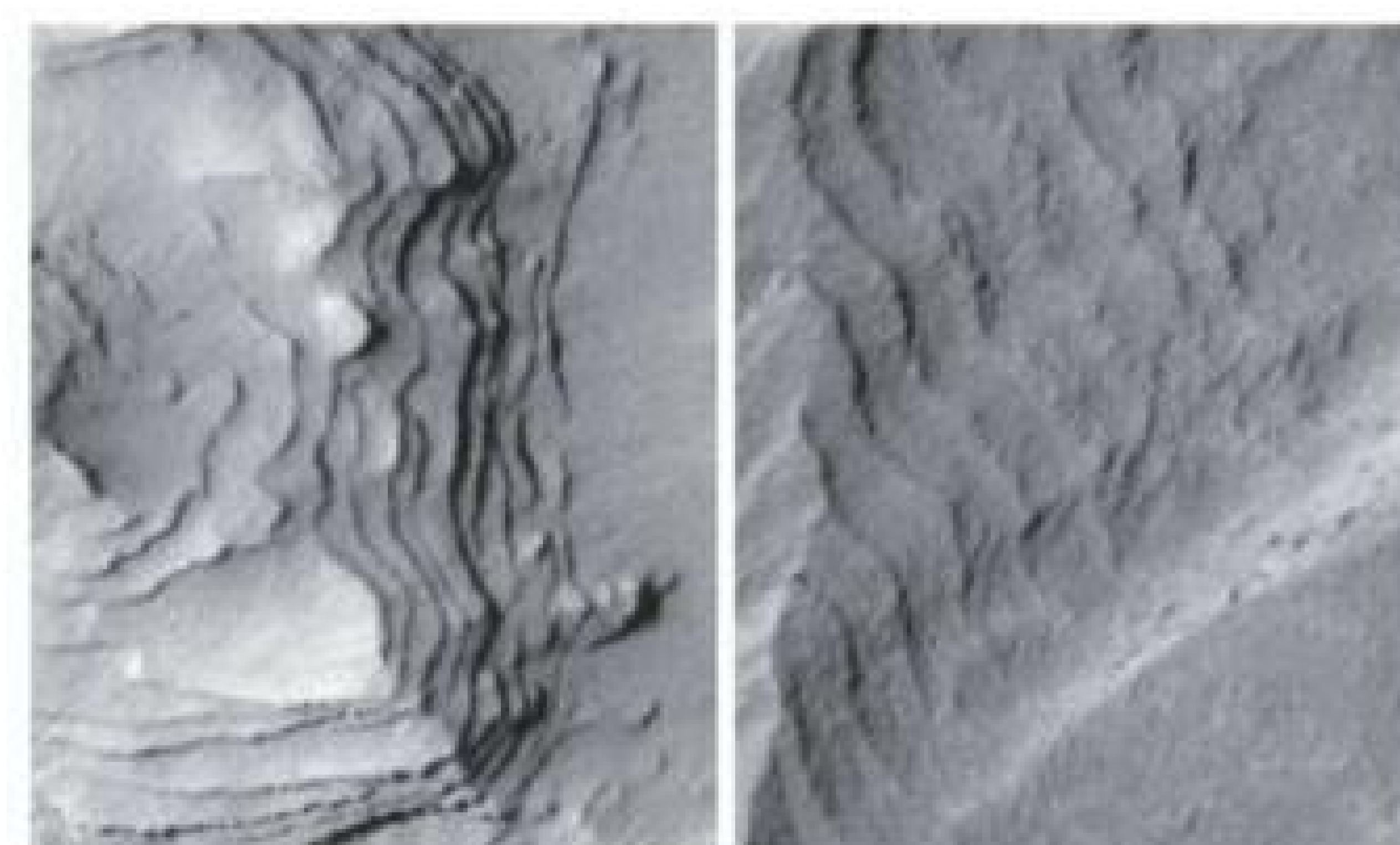
450 Minimum number of California condors required for delisting under the U.S. Endangered Species Act. The population hit 405 on 30 April.

35% Percentage of the U.S. Southern High Plains that, at current groundwater depletion rates, won't sustain irrigation within 30 years, putting crop production at risk, according to a *Proceedings of the National Academy of Sciences* study.

31.6 billion Metric tons of carbon dioxide emitted globally in 2011—a record, the International Energy Agency said on 24 May. A 9.3% increase in emissions from China offset decreases of 1.7% in the United States and 1.9% in Europe.

of sediments in the middle of Gale crater. Mars researchers have no idea how those sediments got there. But a pair of geologists is now suggesting that at least the top third of Mount Sharp is volcanic ash that fell out of the sky surprisingly early in Mars's history.

The Medusae Fossae Formation (left) covers one-third of the martian equato-



rial region, with patches near Gale crater. The formation bears a striking resemblance to layered deposits high on Mount Sharp (right), the researchers noted online 24 May in *Science*. By counting accumulated impact craters, the team has also found that the two deposits were laid down at about the same time: 3.8 billion years ago. When it arrives, Curiosity can probe beneath the thin coating of dust that obscures the deposit to confirm its true nature.

Science LIVE

Join us Thursday, 7 June, at 3 p.m. EDT for a live chat with leading experts on a hot topic in science. <http://scim.ag/science-live>

CREDITS (TOP TO BOTTOM): ARNE HÜCKELHEIM/WIKIMEDIA COMMONS; NASA/HIRISE/UNIVERSITY OF ARIZONA



Frequency splitting. How the core of SKA's high-frequency array will look and (right) a mid-frequency aperture array.



RADIO ASTRONOMY

Telescope Project Splits Array to Avoid Division

A globe-spanning compromise has put the world's biggest scientific instrument on course to become a reality. On 25 May, the member states of the Square Kilometre Array (SKA) radio telescope project ended a competition between two potential hosts by splitting the €1.5 billion telescope between the two sites: one centered in South Africa, the other in Australia. "This keeps everyone onboard and doesn't fragment the community. It's a price worth paying," says Albert Zijlstra, director of the Jodrell Bank Centre for Astrophysics in the United Kingdom.

SKA will be a telescope on a continental scale, spanning thousands of kilometers with thousands of dishes and other antennas. With it, astronomers hope to peer back into the early epochs of the universe when the first stars and galaxies were forming, to study cosmic magnetism, the extremes of gravity around pulsars and black holes, and mysterious dark energy.

Expectation charged the air as representatives from SKA member states gathered at Amsterdam Airport Schiphol for the meeting. Canada, China, Italy, the Netherlands, and the United Kingdom were entitled to vote. Not voting were the potential hosts: South Africa, along with Australia and its partner New Zealand.

Reaching this decision point had been a long and, for the potential hosts, expensive process (*Science*, 30 March, p. 1564). To demonstrate their technical capabilities and commitment to the project, both bid teams have installed considerable infrastructure—roads, power supplies, data links—at their proposed sites and are building prototype "precursor"

arrays: the \$152 million Australian SKA Pathfinder (ASKAP) near Geraldton in Western Australia and the \$190 million MeerKAT in the Karoo region of the Northern Cape.

The competition had a David-and-Goliath aspect. "Australia is right at the forefront [of astronomy]; it has a big reputation," says Zijlstra. South Africa is a relative newcomer but has stressed that SKA will help develop scientific capabilities in southern Africa and inspire young people there to study science. "They're very keen to get this. It's very important for the development of the country," he says.

The SKA members' final decision divided the telescope by frequency: Australia/New Zealand will specialize in low frequencies (below 500 megahertz), while South Africa

higher frequencies altogether: During the first phase of construction, Australia will get 60 new high-frequency dishes to add to the 36 of ASKAP, which will be completed later this year. That is because Australia, in collaboration with Canada and the Netherlands, has been pioneering a new kind of detector to go at the focus of the dishes. Called a phased-array feed (PAF), it enables a telescope to look at more than one part of the sky at once and so greatly speeds surveys. Australia's 96 PAF-equipped dishes will be dedicated to high-speed surveys of the radio sky.

Although the division is not an equal one (in SKA's final configuration, South Africa will have around 3500 antennas to Australia/New Zealand's 400), the bid teams appear happy with the result. "It's an excellent outcome for the project and builds on the infrastructure already in place," says Brian Boyle, director of the Australia/New Zealand team. Justin Jonas, associate director for science and engineering of the South African team, says members are "ecstatic." "Costs aside, there are no negative effects on the scientific capability of the machine, and there could be some positives."

Michiel van Haarlem, interim director of the SKA Organization, which is based in the United Kingdom, says the group will be investigating the cost implications of two sites over the next 6 months. The split decision isn't expected to cause much of a cost increase for phase I of the project (which will put 10% of the full array in place) because the infrastructure already built for ASKAP and MeerKAT will likely be sufficient. Phase II at two sites

will require some duplication, in particular the thousands of kilometers of fiber-optic cable needed to link together each array. But as Jonas points out, adding one member to the collaboration would probably cover the extra cost.

"It's a relief" to have the decision made, says van Haarlem. "Now we can start to actually address the technical implications. We're eager to get on with it."

—DANIEL CLERY

SKA'S TWO-SITE SOLUTION

	Precursors	Phase I: 2016–2020	Phase II: 2020–2024
South Africa	MeerKAT (64 dishes)	190 high-frequency dishes	3000 dishes + 250 mid-frequency aperture arrays
Australia/New Zealand	ASKAP (36 dishes)	60 high-frequency dishes + 50 low-frequency aperture arrays	250 low-frequency aperture arrays

will cover the middle and high frequencies (above 500 MHz). Such a division makes technical sense because the different frequency ranges are processed separately and need different sorts of antennas. "It may not be the most optimal solution, but the project can now concentrate on moving forward," says Michael Kramer of the Max Planck Institute for Radio Astronomy in Bonn, Germany.

Australia is not being shut out of the

ARCHAEOLOGY

Early Dates for Artistic Europeans

For 150 years, Geissenklösterle and other prehistoric caves in southwest Germany have yielded evidence of ancient human culture. The earliest known musical instruments—flutes of bird bone and mammoth ivory—were found in these caves, as were the earliest mythical figurines, both testaments to the creative powers of ancient people. Now new radiocarbon dates put modern humans in Geissenklösterle several thousand years earlier than previously thought. As far back as 42,000 years ago, while the last Neandertals were hanging on in western and southern Europe, modern humans were carving sculptures and making music in central Europe.

The early dates, published online last month in the *Journal of Human Evolution*, also suggest to some researchers that certain artistic behaviors emerged first in Europe rather than Africa. “Very special things were clearly happening in this small area” of Germany, says archaeologist Paul Mellars of the University of Cambridge in the United Kingdom.

Thus the dates bear on three of the most hotly debated questions in human evolution: When and where did *Homo sapiens* first enter Europe; why did the Neandertals go extinct soon afterward; and where did modern human creativity first sprout?

Archaeologists have been working at Geissenklösterle and other caves in the Swabian Jura since the 1860s; archaeologist Nicholas Conard of the University of Tübingen in Germany has led the dig for the past 16 years. These long excavations have revealed a rich trove of artifacts, including eight flutes previously dated to about 35,000 years ago and sophisticated stone tools. The artworks include beads made of ivory and animal teeth; figurines of horses and bison; a half-lion, half-man statuette; and a bizarre figurine of a human female (*Science*, 6 November 2009, p. 784).

Artifacts of this type are typical of the Aurignacian culture, long thought to have been made by the first modern humans who entered Europe. (Some recent research, such as that

based on discoveries at the 45,000-year-old site of Grotta del Cavallo near Apulia, Italy, suggests that modern humans could have been in Europe before the Aurignacian, but there’s no consensus on this suggestion.)

In the new study, Thomas Higham of the University of Oxford in the United Kingdom and colleagues used a new technique called ultrafiltration to remove contaminants from radiocarbon samples, and also used statistical approaches that improve dating accuracy. Using samples from animal bones, the researchers dated the earliest layers at Geissenklösterle to between 42,000 and 43,000 years ago, and the site’s artwork and

extinct. Many researchers had argued that Neandertals learned these talents from incoming moderns. But some, notably archaeologists João Zilhão of the University of Bristol in the United Kingdom and Francesco D’Errico of the University of Bordeaux in France, have contended fiercely that moderns didn’t arrive until about 38,500 years ago, and that the Neandertals invented these art forms independently. The dating was ambiguous enough to prolong the debate. Now Higham’s results knock that contention “out of the water,” Mellars says.

But Zilhão doesn’t see it that way, citing what he says are inconsistencies in the dating at Geissenklösterle, and saying that layers in the cave may have been mixed. “There is a group of colleagues on a mission from God to put modern humans in Europe early

enough to be the authors” of early symbolism, “thereby putting Neandertals back where they ‘deserve’ to be,” Zilhão complains.

The earlier dates may also add weight to Conard’s long-standing assertion that the Aurignacian represented a new stage in the cultural evolution of modern humans. Many researchers think that symbolic behaviors such as the use of beads originated in Africa as early as 100,000 years ago (*Science*, 30 January 2009, p. 569).

But when modern humans entered Europe via what Conard calls the “Danube Corridor,” they faced new challenges, including, possibly, competition with Neandertals as well as the colder climate. Conard argues that this new situation sparked cultural innovation, including four new forms of art found

first in Europe: mythical images such as the lion-man; beads carved in three dimensions rather than made of pierced natural shells and teeth; figurines; and musical instruments. “You wouldn’t expect everything to evolve 100% in Africa,” Conard says, adding that the cultural innovations may have reflected new systems of belief.

Some others agree. “The Aurignacian record for symbolic representation is quantitatively and qualitatively light-years beyond the African record,” says archaeologist Randall White of New York University in New York City. Mellars has argued that the appar-



Artistic explosion? Musical instruments, figurines, and mythical creatures such as the lion-man (right) are first known from Germany, suggesting that some cultural innovations may have taken place after modern humans left Africa.

musical instruments to at least 40,000 years ago. “The results show clearly that artistic expression and complex behavior came to Europe by 42,000 years ago,” Higham says. John Hoffecker, an archaeologist at the University of Colorado, Boulder, says the new dates “are entirely credible, and I think they’ll be widely accepted.”

That means that the Aurignacians were making art while Neandertals were still in Europe. Neandertals themselves began making personal ornaments and other symbolic objects in France and Spain about 40,000 years ago, shortly before they went

ent explosion of symbolic activity in Europe reflects social networking among denser populations (*Science*, 29 July 2011, p. 623). And some researchers suggest that the lack of independent art in Neandertals was part of a package of small population sizes or other disadvantages that sped their extinction.

But others aren't ready to concede that something special happened when moderns reached Europe. Anthropologist Alison

Brooks of George Washington University in Washington, D.C., has long argued that the supposed cultural explosion in Europe was a "revolution that wasn't" (*Science*, 4 May, p. 530). She notes that recent dating of paintings at the Apollo 11 rock shelter in Namibia—which include a half-human, half-animal depiction—puts them at 30,000 years ago, in the ballpark of some of the oldest cave paintings in Europe. "The people

who left Africa already had these capabilities but either didn't express them or, more likely, the poor organic preservation and lack of deep caves in Africa" has made it difficult to find such evidence, Brooks says. Hoffecker agrees: "It doesn't make sense to me that the capacity for visual art and music in modern humans would evolve or develop independently in different places and times."

—MICHAEL BALTER

ASTRONOMY

Creative Deal Gives NASA Telescope New Lease on Life

Why throw away a perfectly good space telescope when its best scientific days might lie ahead? That thought was on the mind of physicist Christopher Martin of the California Institute of Technology (Caltech) in Pasadena last fall as NASA officials prepared to turn off the Galaxy Evolution Explorer (GALEX).

The telescope had already spent 8 years in orbit, surveying the sky at ultraviolet wavelengths. Originally intended to last 29 months, the mission was not eligible for any more extensions, and NASA was scheduled to send a "kill command" to the instrument on 19 December 2011, shutting it off for good. But Martin, principal investigator on the project, had other ideas. Now the telescope has a fresh lease on life, thanks to a first-of-a-kind arrangement between the federal government and a research university.

NASA has loaned the satellite to Caltech under a rarely used legal provision that allows the institution to operate the instrument as its own without assuming any liability for it. The operating costs—about \$1.2 million a year—will be the responsibility of Caltech, which has so far raised about half that amount through contributions from the university and other institutional donors, along with personal pledges to the tune of \$40,000 from Martin and his colleagues.

"From our standpoint, this will be a great use of a public asset," says Jaya Bajpayee, the GALEX program executive at NASA headquarters. "One of the positives is that we have set up a precedent that we can apply to other missions in the future." A NASA spokesperson told *Science* that the agency would be open to similar arrangements for two other space telescopes that were recently decommissioned: the Wide-Field Infrared Survey Explorer, which was put on standby mode in

February 2011, and the Rossi X-ray Timing Explorer, which was turned off in January.

The effort to prolong GALEX's life began one evening last November over dinner between two longtime friends: Martin and his Caltech colleague, Shrinivas Kulkarni. It was obvious to them that the telescope still had a lot of good science left in it. In fact, Martin had come to comprehend only months earlier that GALEX is capable of a broader range of studies than its original task of surveying other galaxies.

In early 2011, realizing that the mission's end was in sight, Martin and his colleagues began pushing the instrument beyond its limits. The astronomers pointed it at the plane of the Milky Way. "We thought the data quality would be poor because we thought the electronics would not be able to handle the high load of photons falling upon the detectors from this bright region of the sky," Martin says. To the surprise and delight of the GALEX team, the telescope performed very well. Focusing on our own galaxy will enable the astronomers to study the evolution of stars rather than galaxies.



Promoter. Martin hopes to raise more money for Galex.

Martin and Kulkarni hatched a plan to keep GALEX going using private funds. Kulkarni says he was shocked when he learned that it would cost only \$100,000 a month to continue the mission. "It seemed that for the lack of a hair clip, we would lose a beautiful hat," he says.

The two astronomers, along with a few other colleagues, began the fundraising effort by pledging several thousand dollars of their own money. Martin pledged \$6000 from his savings. His wife wasn't thrilled, he jokes, but she told him "You do what you got to do." Caltech offered to help out, and so did a consortium of other universities.



Extended. Galex will now focus on regions within the Milky Way.

In December, after receiving the backing of Caltech administrators, Martin approached NASA to ask if Caltech could take ownership of the telescope. NASA and Caltech officials began discussing how to make the transfer. A straightforward transfer would have required Caltech to assume liability for damages if GALEX crashed into another satellite or caused any damage upon reentering Earth's atmosphere several decades into the future. That's not something Caltech could afford to take on, Martin says.

NASA officials came up with a solution. Under a Space Act Agreement signed between NASA and Caltech, "the satellite could be loaned to Caltech such that Caltech would be responsible for operating it and acquiring further data, but liability would not have to be transferred," Martin explains. The deal was sealed on 14 May.

The funds raised so far will allow Caltech to operate the telescope until October. Martin and his colleagues hope to keep GALEX going a lot longer, and intend to submit proposals to the National Science Foundation and other federal agencies. They also plan to continue seeking private donations.

—YUDHIJIT BHATTACHARJEE



PSYCHIATRY

Criticism Continues to Dog Psychiatric Manual as Deadline Approaches

Less than a year before a new edition of the diagnostic reference for psychiatric illnesses is to be released, the controversy it has generated continues. The *Diagnostic and Statistical Manual of Mental Disorders*, now in its fifth edition (*DSM-5*), is the most influential catalog of mental and behavioral disorders, and the 13-year revision process has sparked much heated discussion as psychiatrists decide what new diagnoses to add and which existing ones to change or eliminate (*Science*, 12 February 2010, p. 770).

A lot is riding on this tome. Psychiatrists and psychologists use its descriptions of hundreds of conditions to diagnose their patients. Health insurers use it to decide which treatments to cover. Pharmaceutical companies and academic researchers use it to set research priorities.

Its publisher, the American Psychiatric Association (APA), insists that the new edition improves on its predecessor and is on track to be released next May. Critics say they're bracing for a train wreck.

"They really believe in what they're doing, but they're wrong," says Allen Frances, an emeritus professor of psychiatry at Duke University in Durham, North Carolina, and a dogged critic of *DSM-5*.

Frances oversaw the last major edition of the manual, *DSM-IV*, first published in 1994. Frances—and he's not alone—complains that proposed revisions that aim to identify earlier stages and milder forms of mental illness will have serious unintended consequences. Critics argue that *DSM-5* will turn ordinary angst into illness and result in millions of people who don't need medication taking drugs with major side effects. "The sickening of society is moving apace," says Frank Farley, a psychologist at Temple University in Philadelphia, Pennsylvania, and a past president of the American Psychological Association.

At this year's APA meeting, held last month in Philadelphia, *DSM-5* leaders announced that they were backing off from two of the most controversial new diagnoses: a precursor to schizophrenia and a combination of mild anxiety and depression. Both will be relegated to "Section III," an appendix of *DSM-5* for proposed conditions that require further study. But several other proposals continue to cause a stir, including a new childhood disorder involving angry outbursts. "That's currently the most dangerous diagnosis," Frances says.

Beneath the bluster lie profound ques-

tions about human emotion, cognition, and behavior. Where is the line between healthy and unhealthy? Is a person's mental well-being influenced more by innate biology or life experience? Why do so many people with problems not fit into existing diagnoses? How can the risk of overmedicating healthy people be balanced against the risk of sick people going untreated? It's no wonder remaking the manual hasn't been easy.

To include or not to include

When planning for *DSM-5* first began in 1999, its leaders hoped to tap advances in neuroscience and genetics to create a taxonomy of mental illness that better carved nature at its joints (*Science*, 31 October 2003, p. 808). That didn't happen to the extent they had hoped. "We could not construct a diagnostic classification on the basis of neuroscience findings at this point," acknowledges David Kupfer, a psychiatrist at the University of Pittsburgh School of Medicine in Pennsylvania and the chair of the *DSM-5* committee overseeing the revisions. But Kupfer says biology is still at the core of *DSM-5*, which will group disorders thought to involve similar genetics or neural circuitry and mention biological mechanisms in the text describing disorders.

DSM-5 aims to close the gaps between diagnoses by adding some new ones and altering the definitions of some existing ones. Kupfer estimates, for example, that up to 50% of people with eating disorders and 20% of those with mood disorders don't meet *DSM-IV* criteria for a specific diagnosis. The new edition also tries to help clinicians evaluate symptoms along a continuum instead of ticking a yes-or-no box, and it acknowledges that many symptoms occur in multiple disorders. A new measure of suicide risk, for example, can be used regardless of a patient's diagnosis.

DSM-5 also emphasizes the progression of disorders over time. There's a growing recognition that serious psychiatric problems are often preceded by milder symptoms that don't necessarily fit the criteria for any existing disorder, Kupfer says: "What we are dealing with is, what are the prodromes of these psychiatric conditions, and what are the ways we can think about early intervention and perhaps even prevention?"

One of the recently scrapped proposals, attenuated psychosis syndrome (originally called psychosis risk syndrome), was the controversial poster child for this approach. Research in the past 2 decades has established that people who develop schizophrenia often experience milder delusions,

CREDIT: "PSYCHOSIS" BY AMBER CHRISTIAN OSTERHOUT, CREATOR OF THE GAINING INSIGHT CAMPAIGN, WWW.GAINING-INSIGHT.COM

hallucinations, and disordered speech years earlier, says William Carpenter, a psychiatrist at the University of Maryland School of Medicine and chair of the *DSM-5* psychotic disorders work group. More importantly, Carpenter says, there's evidence that early interventions—such as dietary supplements of omega-3 fatty acids—can prevent a slide into full-blown psychosis.

But others worried that the new diagnosis would be too broadly applied. “A lot of quirky kids I see would have been looked at as having prodromal schizophrenia, and their parents would have been terrified, and they would have been put on drugs they don't need,” says Gabrielle Carlson, director of child and adolescent psychiatry at Stony Brook University School of Medicine in New York. She is not involved with the *DSM-5* revisions. Says Frances: “You would have had kids who would never become psychotic taking drugs that can make them fat, give them diabetes and heart disease, and maybe, shorten life expectancy.”

Ultimately, the diagnosis was relegated to Section III not because of these concerns, but because field trials sponsored by APA were too small to determine whether clinicians could diagnose it reliably, Carpenter says.

Full results from field trials have not yet been made public, but preliminary analyses presented at the recent APA meeting suggest that several of the new diagnoses—and even some old standbys like major depression and generalized anxiety disorder—got surprisingly low scores on a measure of how reliably different clinicians arrive at the same diagnosis for a given patient, prompting Frances and other *DSM-5* critics to question the validity of the field trials.

Low reliability scores are what relegated the newly proposed mixed anxiety-depression diagnosis to Section III, says Jan Fawcett, a psychiatrist at the University of New Mexico, Albuquerque, and chair of the *DSM-5* work group on mood disorders. This diagnosis was intended for people who show signs of both depression and anxiety, but whose symptoms are too mild to merit a diagnosis of either major depressive disorder or generalized anxiety disorder. “This is seen a great deal in primary care,” Fawcett says.

Frances predicts that mixed anxiety-depression would have become the most common psychiatric diagnosis if it had survived. “It would have leaked into the aches and pains of everyday life, the worries and sadness that all of us feel,” he says. “It's good that it's off the table.”

Still undecided

Among the proposed revisions still in the running, few have generated more public backlash than the proposal to remove the clause in *DSM-IV* that prevents people from receiving a diagnosis of major depression within 2 months after the death of a loved one. “We're being criticized for medicalizing normal grief,” Fawcett says. But he argues that a bereaved person could still have major depression. His work group is



developing language to help clinicians distinguish depression from grief, whether it's caused by a death or another personal catastrophe. “We think there's a way to distinguish the two,” Fawcett says.

Another proposed diagnosis still generating debate is disruptive mood dysregulation disorder for children prone to outbursts that go beyond the usual childhood tantrums. Called temper dysregulation disorder with dysphoria when it was first introduced, it was intended to

stem what many researchers and clinicians see as an overdiagnosis of bipolar disorder in children (*Science*, 5 March 2010, p. 1192). But the new diagnosis has gotten a cool reception. A better solution, Carlson says, would have been to consider explosive outbursts as an optional add-on to other diagnoses, as in attention deficit disorder *with* explosive behavior. Creating a standalone diagnosis for temper outbursts is like creating a diagnosis of “fever of unknown origin,” she says, because it doesn't tell you anything about the underlying cause or how to treat it.

Disruptive mood dysregulation disorder is one of several proposed diagnoses flagged as lacking scientific backing in a letter posted online by members of the American Psychological Association's humanistic psychology division. The letter, which offers a broad critique of *DSM-5*, has been signed by more than 13,500 individuals and several dozen professional organizations working in mental health. Several of the letter's authors, including Farley, wrote again to the APA in January to call for an independent scientific review, by an impartial party such as the Institute of Medicine, of some of the more controversial *DSM-5* proposals. Frances and others have made similar pleas, but Kupfer says there are no imminent plans to do so.

The psychologists' letter also argues that the push to ground *DSM-5* in biology gives short shrift to social and cultural factors that contribute to mental illness. “We are social animals, and a lot of our problems arise out of the social context of our lives,” Farley says. Psychologists and other mental health professionals often emphasize these nonmedical factors more than psychiatrists do, and Farley says they should have more of a say in establishing diagnostic guidelines. Toward that end, he and colleagues are planning a meeting next year that will bring together “a much broader coalition” of experts to discuss alternatives to the *DSM* format. “Diagnosing mental illness is just too big a job, with too many implications, for any one profession to be in charge of it,” Farley says.

The third and final period for public comment on *DSM-5* ends 15 June. The proposed revisions will undergo internal reviews before being sent to the board of trustees for approval. The final draft will go to press by the end of the year and be officially released next May at APA's annual meeting in San Francisco. To think that will be the end of these debates would be, well, crazy.

—GREG MILLER

See *Science's* previous series on the proposed *DSM-5* revisions at <http://scim.ag/draftDSMV>. The official *DSM-5* Web site is at <http://www.dsm5.org/>.

Mysteries of Astronomy

What Is Dark Energy?

Fourteen years ago, the discovery of dark energy rocked astronomy. Two teams of astronomers and astrophysicists studied distant stellar explosions called type Ia supernovae to measure how the universe has expanded over its 13.7-billion-year lifetime. They expected that the expansion would be slowing as galaxies pull toward one another with their gravity. To their shock, they found that the expansion is accelerating as if some bizarre “dark energy” is stretching space. The nature of dark energy is now perhaps the most profound mystery in cosmology and astrophysics. And it may remain forever so. “Part of the mystery is that we have no clue whether we will be able to find an answer,” says Simon White, an astrophysicist at the Max Planck Institute for Astrophysics in Garching, Germany.

Dark energy could be one of three things. It could simply be a property of empty space itself. Einstein’s theory of gravity, known as general relativity, allows for just such a “cosmological constant” that would be a property of the vacuum and would stretch space. Or, in a radically different alternative, dark energy could be a new type of force field that occupies space, much as air fills a balloon. That second alternative is known as “quintessence.” Finally, dark energy could be an illusion, a sign that scientists’ understanding of gravity as encapsulated in general relativity isn’t quite right.

To sort through the possibilities, scientists hope to answer one key question: How does the density of dark energy vary as space expands? If dark energy is a cosmological constant, then—true to that name—its density should remain constant. If dark energy is something within space, then it should grow more dilute as space expands. It all comes down to measuring the ultrasimple “equation of state” of dark energy and whether a single parameter, denoted w , equals -1 , for a cosmological constant, or something like -0.9 for a quintessence.

To probe dark energy, astronomers can generally make two types of measurements. The first seeks to reconstruct more precisely the history of the universe’s expansion. For example, type Ia supernovae all pump out the same amount of light, so observers can tell how far away one is by how bright it appears in the sky. At the same time, the universe’s expansion causes each supernova to speed away from Earth and stretches its light to longer, redder wavelengths. That “redshift” reveals when a supernova went off. So by measuring the distances to many supernovae at various redshifts, scientists should be able to deduce how space stretched over cosmic time, enabling them to measure the equation of state and whether that equation has changed. Researchers can play a similar game with traces of sound waves from the infant universe, called baryon acoustic oscillations, that imprinted themselves on the afterglow of the big bang and the distribution of the galaxies.

Online

sciencemag.org

S Podcast interview
with author Robert
Coontz (http://scim.ag/pod_6085).

The second type of measurement seeks to detect dark energy’s effects on the formation of the largest structures in the universe. For example, galaxies gather in huge clusters as their gravity pulls them together. But space-stretching dark energy should counteract gravity and slow such clustering. So researchers can probe dark energy and its equation of state by simply counting clusters of various sizes, as they have already begun to do. Similar clues may come from charting the vast web of mysterious dark matter thought to span intergalactic space (see p. 1091). The strands in the web form as gravity draws dark matter together, but dark energy’s space-stretching effect should

Endless mysteries lurk in the depths of space. To pare the list down to eight—now, there’s a challenge. In deciding what to include in this section, *Science*’s news staff teamed up with *Science* Associate Editor Maria Cruz and consulted researchers on the Board of Reviewing Editors and elsewhere. From the outset, the team decided that true mysteries must have staying power (as opposed to mere “questions” that researchers might resolve in the near future). Some of the finalists are obvious shoo-ins; others have received less of the popular limelight. The final selection spans the entire history of the universe on scales ranging from our sun and its planetary system to the entire cosmos. Each mystery is sure to be solved largely through astronomical observations—if it is solved: In at least one case, experts aren’t sure that a seemingly simple question will ever be answered.

—ROBERT COONTZ

slow that process. Astronomers can trace the evolution of the web by measuring how the gravity from the unseen strands bends light rays from more-distant galaxies and distorts their images—an effect known as weak lensing.

To maximize their progress, astronomers hope to play these experimental tools off of one another. For example, starting later this year, 120 researchers working with the Dark Energy Survey (DES) plan to use the 4-meter Blanco telescope at the Cerro Tololo Inter-American Observatory in Chile to study 200 million to 300 million galaxies, catalog 100,000 galaxy clusters, and spot 4000 supernovae. That information should enable them to use all four techniques to measure dark energy's equation of state.

Such a multipronged attack might enable scientists to answer the most basic question: Does dark energy really exist, or is the theory of gravity lacking? For example, if studies of supernovae and galaxy clusters yield inconsistent results, then that would be a signal that the real problem lies with the theory of gravity, says Josh Frieman, an astrophysicist at Fermi National Accelerator Laboratory in Batavia, Illinois, and the nearby University of Chicago and a founder of the \$50 million DES. "I don't know how far [this strategy] would get us down the road, but at least

it would get us down the road," Frieman says. "Right now, we're stuck at the crossroads."

Astronomers are planning even bigger efforts to probe dark energy. Late this decade, the European Space Agency plans to launch a \$570 million space telescope called Euclid to study dark energy primarily through measurements of baryon acoustic oscillations and weak lensing. On the ground, astronomers in the United States hope to build the Large Synoptic Survey Telescope, a proposed \$465 million, 8.4-meter telescope that would take as one of its primary missions studying dark energy using all four techniques.

Wow ... The Blanco Telescope in Chile, which will conduct the Dark Energy Survey, silhouetted against the Milky Way.



Dark energy might never reveal its nature, however. Data from supernovae, galaxy clusters, and baryon acoustic oscillations currently fix w at -0.98 , give or take 10%, a result consistent with either a cosmological constant or quintessence. But theorists can't predict exactly how far from -1 w should be if dark energy is in fact a form of quintessence. So if, for example, more data yield a result for w of -0.99 plus or minus 1%, that number would still be ambiguous: consistent with either a cosmological constant or quintessence. And some scientists

think such a march toward eternal uncertainty is likely. "I think it's a safe prediction that w will turn out to be -1 to within 1%, to within 0.1%, etc.," says David Gross, a theoretical physicist at the University of California, Santa Barbara.

Still, scientists remain optimistic that nature will cooperate and that they can determine the origins of dark energy. "I tell my observer friends that it's worth spending a decade of their careers trying to do it," says Rocky Kolb, a cosmologist at the University of Chicago. "There's a chance to take a big step forward."

—ADRIAN CHO

How Hot Is Dark Matter?

For decades, astronomers have thought that some unseen "dark matter" provides the gravity that holds the galaxies together. Scientists still don't know what dark matter is, but that could soon change. Within years, physicists might be able to detect particles of the stuff. Even if they can't, astronomers and astrophysicists may be able to narrow in on dark matter's most basic properties by astronomical means alone.

In particular, studies of runty "dwarf galaxies" might test whether dark matter is icy cold as standard theory assumes, or somewhat warmer—essentially a question of how massive particles of dark matter are. "Maybe the mass is in a range in which we can measure it by astronomical means," says Marc Davis,

a cosmologist at the University of California, Berkeley. "That would be astounding."

The first signs of dark matter came in 1933, when Swiss astronomer Fritz Zwicky found that the galaxies in the Coma Cluster zing through space so fast that their own gravity shouldn't hold them together. Some invisible matter must provide the extra gravity that keeps the cluster intact, he reasoned. His wild idea got a big boost in the 1970s when American astronomer Vera Rubin and others found that the stars in individual galaxies also whirl around too fast to be held in by their mutual gravity.

However, the strongest evidence for dark matter has come from recent cosmological measurements of the universe's origins.

For example, the afterglow of the big bang, or cosmic microwave background, varies in temperature across the sky, and the tiny variations reflect the sloshing of ordinary matter and dark matter in the infant universe. In 2003, NASA's spaceborne Wilkinson Microwave Anisotropy Probe measured those variations and showed that 83% of all matter in the universe is dark matter.

Cosmologists bolstered the case for dark matter by mapping the galaxies to determine the universe's large-scale structure. For example, starting in 1997, astronomers with the Two-Degree-Field Galaxy Redshift Survey, based at the Siding Spring Observatory near Coonabarabran, Australia, mapped 220,000 galaxies and found that they

were arrayed in vast threads and sheets.

Those observations jibe with the results from another key advance: high-precision computer simulations of how the universe evolved. Those simulations show that a vast web of filaments and clumps of dark matter formed as the stuff coalesced under its own gravity. The galaxies formed as the clumps, or “halos,” drew in ordinary matter that formed stars. The simulations reproduce the statistical distributions of the galaxies so well that the scenario is now cosmology’s standard model.

Now, however, some researchers have begun to wonder if the model is not quite right. To reproduce the weblike structure, theorists assume that dark matter is “cold”—in other words, composed of slow-moving, heavy particles between one and 1000 times as massive as a proton. But cold dark matter simulations run into their own problems.

For example, they produce myriad tiny halos. If all these halos were to collect enough gas to form galaxies, then the Milky Way galaxy should be surrounded by thousands of dwarf galaxies. Instead, astronomers have spotted about 20 dwarf galaxies, suggesting that the little halos don’t exist. That discrepancy is known as the “missing satellites problem,” and it challenges the prevailing theory. “If we can prove

these 100,000 halos are not there, then we can rule out cold dark matter,” says Julio Navarro, an astrophysicist at the University of Victoria in Canada.

Cold dark matter simulations also predict that the density of a dark matter halo should peak in a “cusp” at its center. Instead, observations suggest that galaxies have broader cores in which dark matter is distributed more homogeneously. Known as the “core-cusp problem,” this discrepancy also challenges the prevailing cold dark matter theory. However, in a real galaxy, gravitational tugging between dark and ordinary matter could blur the cusps.

If dark matter were slightly warmer than assumed, however, the smallest halos wouldn’t form and the cusps would smooth out. So even leaders in the field say that dark matter might be warmer than presumed and its particles lighter, each with a mass a few millionths that of a proton. “I have a huge investment in cold dark matter—my whole career and something like 300 papers—but it could be wrong,” says Carlos Frenk, a cosmologist at the University of Durham in the United Kingdom.

Scientists have various ideas about how to take dark matter’s temperature. Frenk says the real issue with the missing satellites is not the tiniest, most numerous halos, as it’s easy to think of reasons they might accumulate too little ordinary matter to light up as galaxies and so remain hidden. The real problem, he says, is that the Milky Way has only three larger satellite galaxies with masses a billion times that of the sun, whereas cold dark matter simulations predict there should be about 10. That prediction depends on the total weight of the Milky Way, Frenk says, so the best way to put cold dark matter to the test is to find a way to

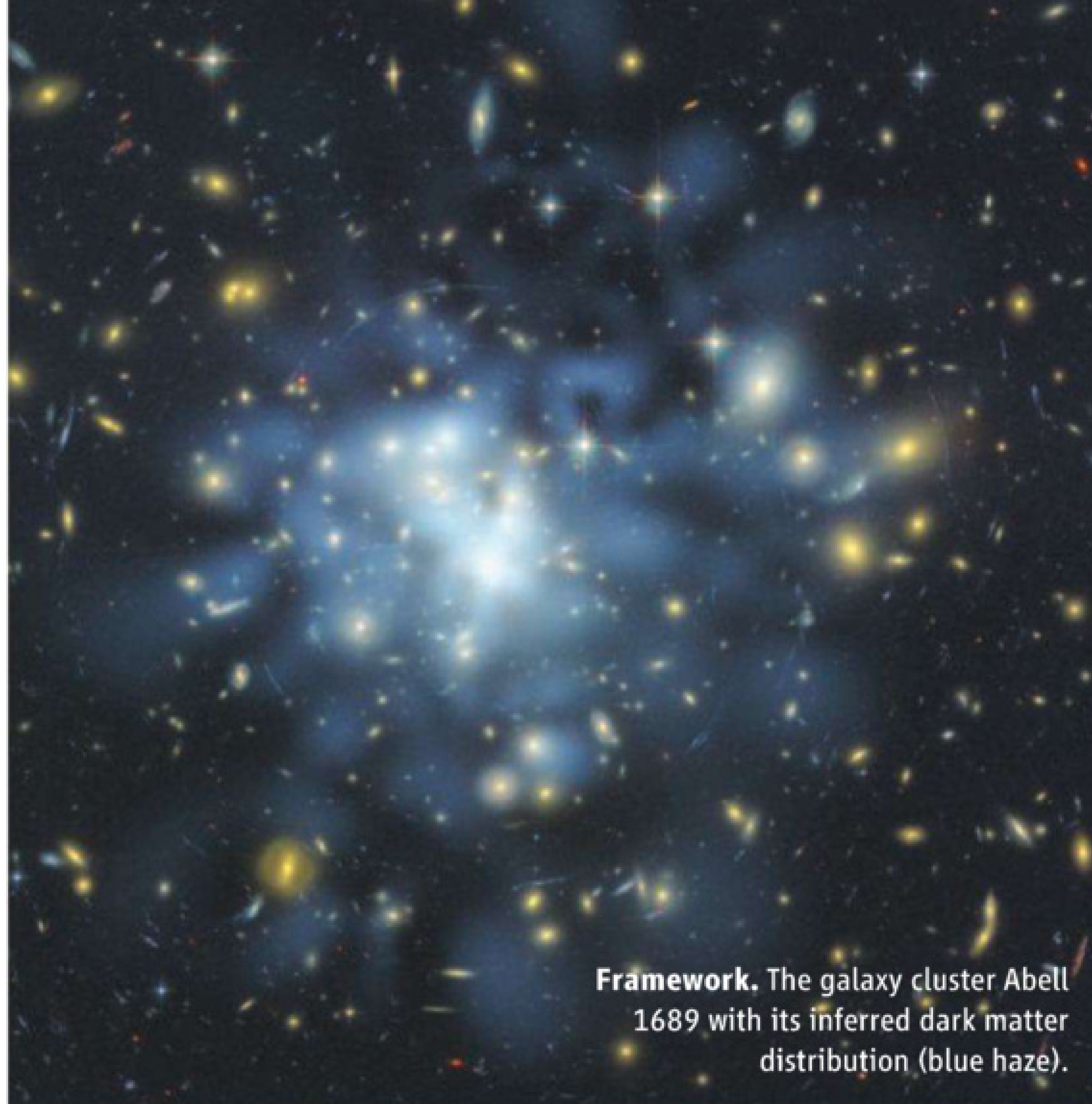
precisely weigh the whole galaxy.

In contrast, Navarro says the key to probing the temperature of dark matter is to tackle the core-cusp problem by studying the smallest, faintest dwarf galaxies surrounding the Milky Way. Such galaxies can have fewer than 100,000 stars and are nearly pure dark matter. So by tracing the motions of their stars, astronomers might deduce the structure of nearly pure dark matter halos and compare it with the prediction of simulations.

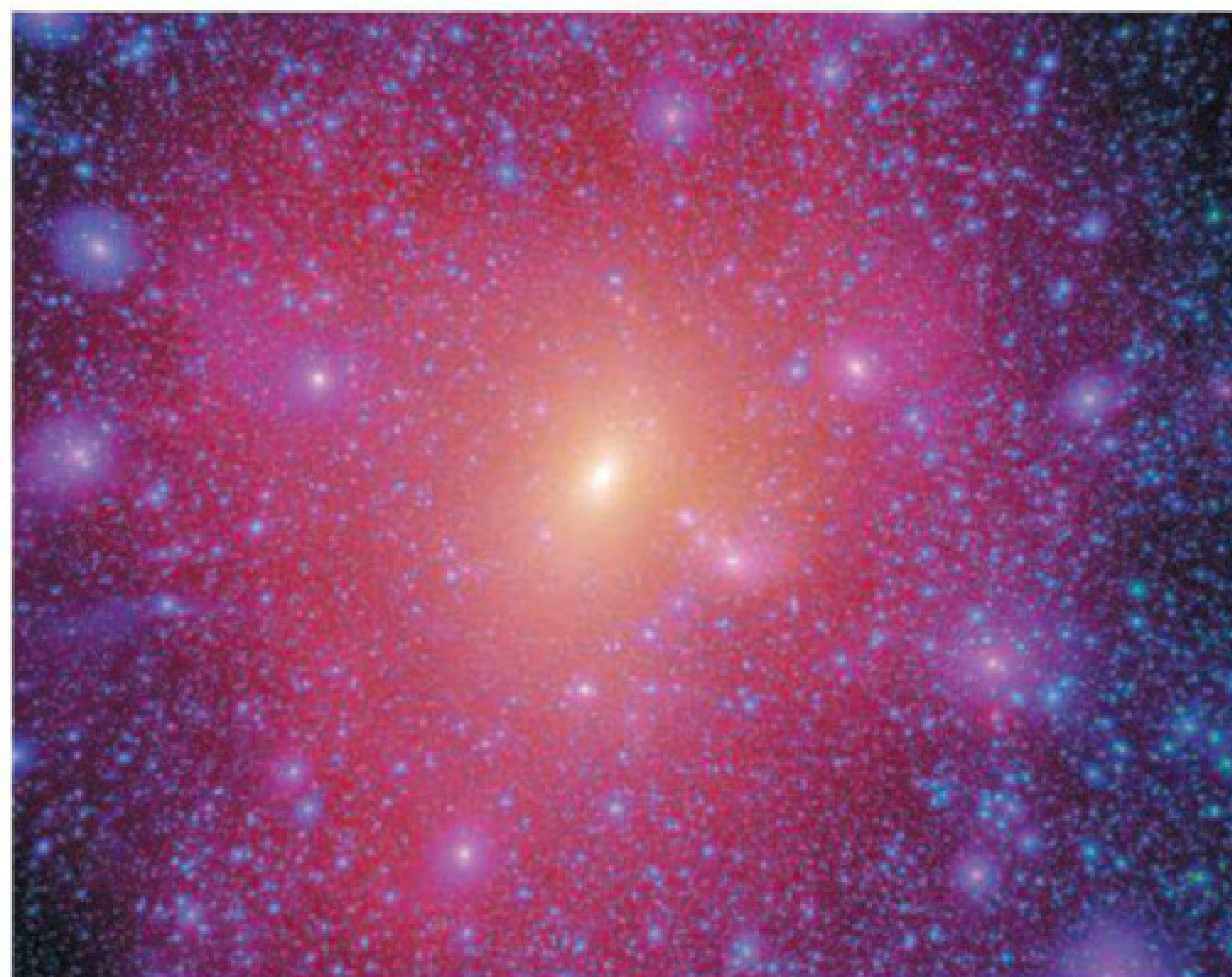
Meanwhile, particle physicists might soon detect dark matter directly. A theory called supersymmetry predicts the existence of weakly interacting massive particles (WIMPs) that weigh a few hundred times as much as a proton and would be ideal candidates for cold dark matter. Detectors deep underground could spot WIMPs floating around. Or the world’s largest atom smasher, the Large Hadron Collider (LHC) in Switzerland, could blast them into existence.

Such a discovery would greatly bolster the case for cold dark matter. But it might not change the way astronomers probe the stuff’s behavior. “If the LHC announced tomorrow that it had found something that could be the dark matter, what I would do on that day would be pretty much the same,” says Kristine Spekkens, an astronomer at the Royal Military College of Canada in Kingston, who is trying to deduce the structure of galaxy halos. If physicists don’t detect WIMPs, then astronomical observations would remain the only way to study dark matter. Apparently, that wouldn’t necessarily stop progress.

—ADRIAN CHO



Framework. The galaxy cluster Abell 1689 with its inferred dark matter distribution (blue haze).



Just like home. An image from the Aquarius simulation of the dark matter halo of a galaxy the size of the Milky Way. The myriad clumps suggest our galaxy should have many more satellite galaxies than it does.

CREDITS (TOP TO BOTTOM): NASA, ESA, E. JULLO (JPL/LAM), P. NATARAJAN (YALE) AND J. P. KNEIB (LAMI); COURTESY V. SPRINGEL, HITS/UNIVERSITY OF HEIDELBERG

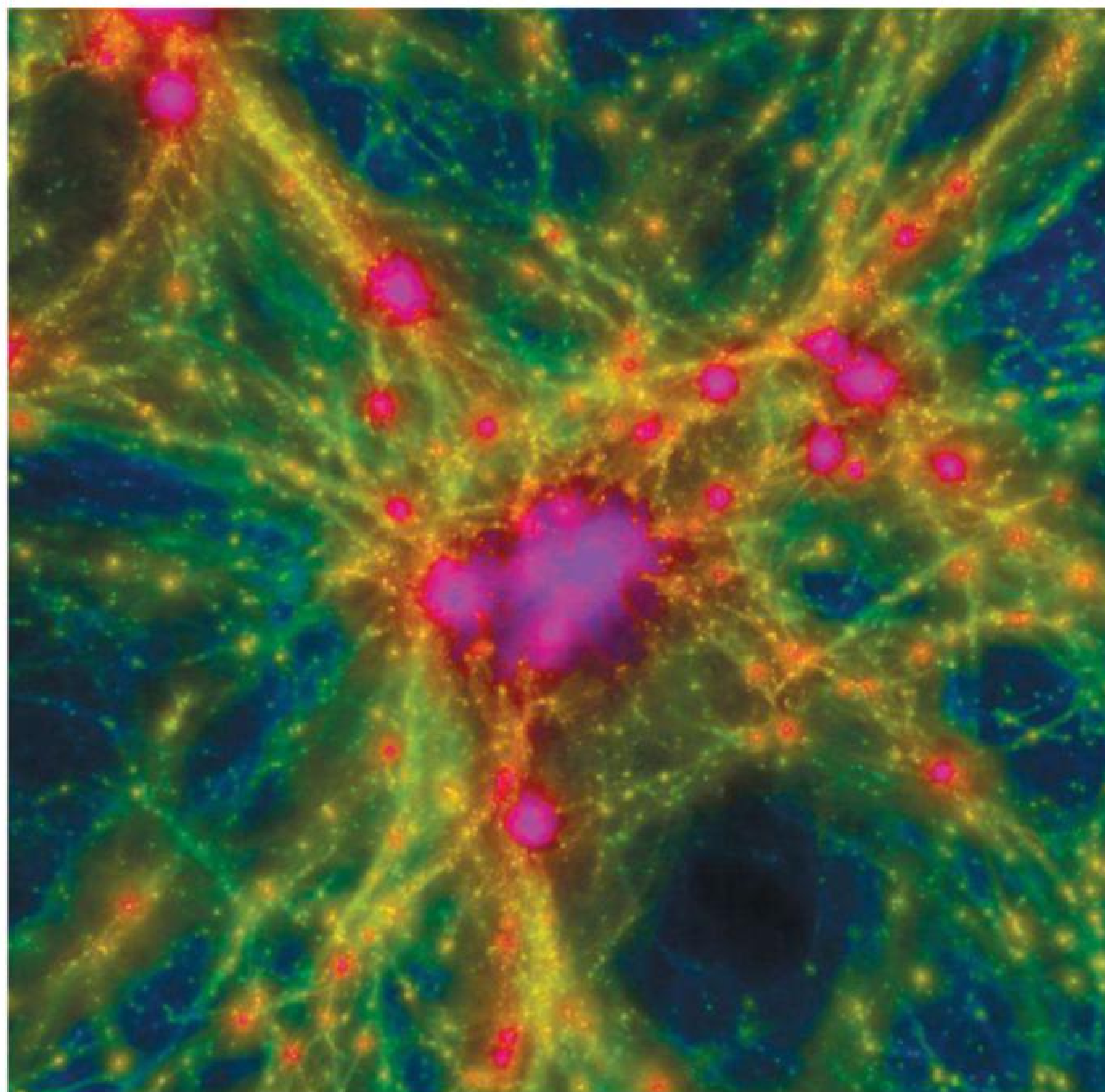
Where Are the Missing Baryons?

To describe the universe, you need to know what's in it and where the components reside. So far, astronomers are a long way from completing that inventory. It's not just that they can't pin down dark energy and dark matter, the two invisible components that make up 95% of the cosmos (see pp. 1090 and 1091). More than half of the remaining 5%—"baryonic matter," the ordinary atoms and ions that make up stars, planets, dust, and gas—remains unaccounted for as well.

Baryonic matter is so called because its main ingredients, protons and neutrons, belong to a class of particles called baryons. Cosmologists have calculated the density of baryons in the primordial universe from measurements of the cosmic microwave background—the faint afterglow of the big bang. The universe has changed a lot in the 13.7 billion years since the big bang, but its original complement of baryons should still be with us today.

As astronomers count baryons from the early universe to the present day, however, the number drops mysteriously, as if baryons were steadily vanishing through cosmic history. By analyzing light from distant quasars to measure the amount of the hydrogen isotope deuterium in ancient baryonic clouds, astronomers can infer that nearly all of the universe's original baryons were still around 10 billion years ago. By contrast, when they take stock of the nearby (and thus more recent) universe—adding up the mass of stars, gas, and everything else they can detect—the inventory barely reaches half of what it should be.

Although galaxies seem like the weightiest things in the universe, they account for only 10% of its baryonic mass. Another 10% comes from warm gas in the space between



Too hot to be seen. The yellow regions in this simulation of a pocket of the universe represent the warm-hot intergalactic medium, which may make up the bulk of missing baryons.

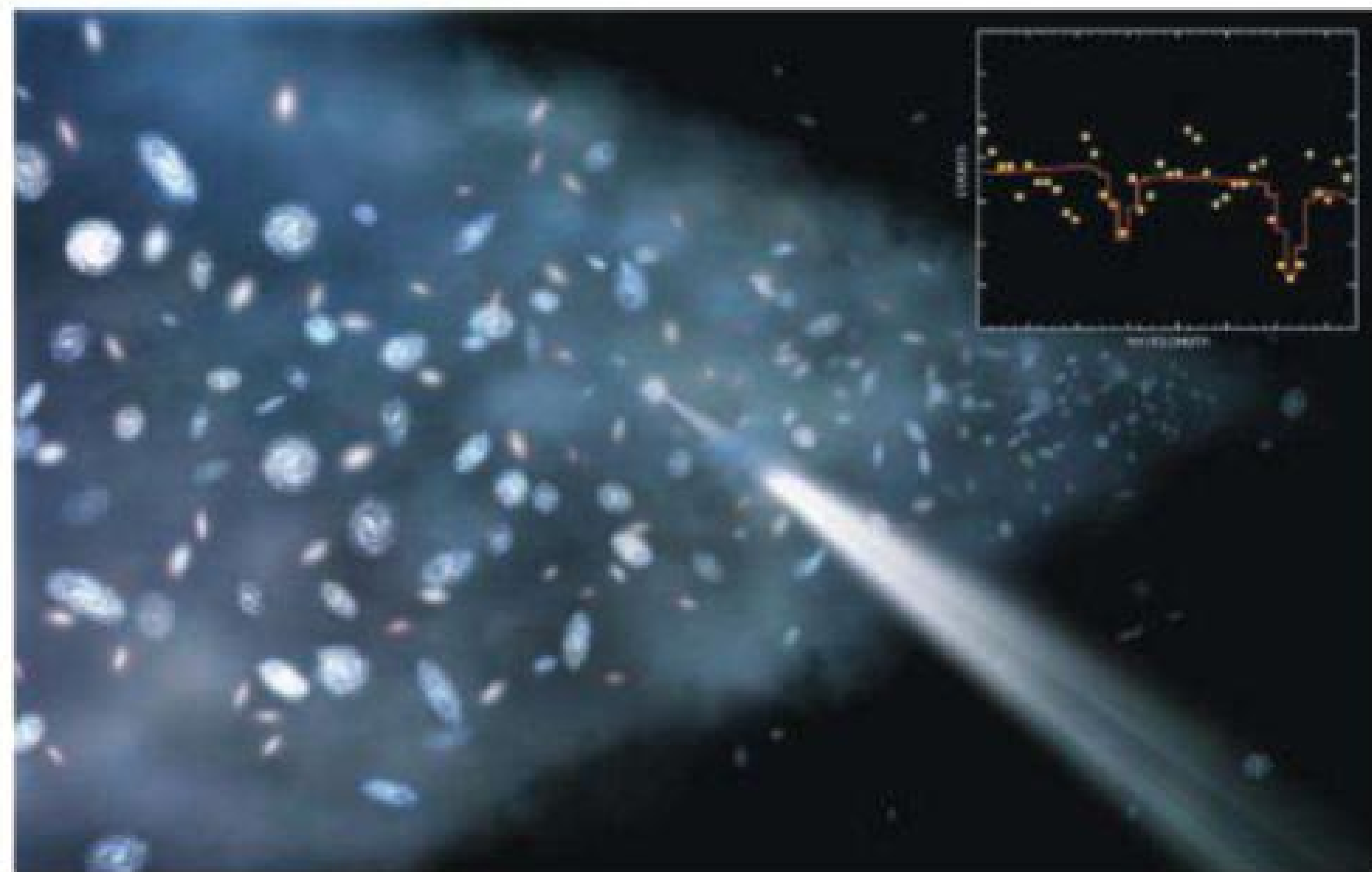
galaxies. A further 30% or so is present in blobs of cold gas in intergalactic space.

Astrophysicists suspect that the missing 50% lies between galaxies in the form of a diffuse, hot plasma just a millionth as dense as the gas found between stars. Astronomers call this material the warm-hot intergalactic medium (WHIM). The medium's temperatures are so high, ranging from 100,000 to 10 million K, that the material in it is highly ionized and can absorb and emit radiation only at far-ultraviolet or low-energy x-ray wavelengths. Because of that finickiness and WHIM's low density, light passing through the medium does not produce spectral lines of the kind that astronomers rely on to spot and study

interstellar gas. As a result, detecting WHIM remains a challenge.

To find WHIM, astronomers have been searching spectra of light passing through intergalactic space for lines corresponding to ions that are thought to survive in the WHIM and attest to its presence. The markers include Neon VIII (neon atoms stripped of eight electrons) and Oxygen VI (oxygen stripped of six electrons). In a paper submitted recently to *The Astrophysical Journal*, J. Michael Shull and others at the University of Colorado, Boulder, claim to have detected WHIM through Oxygen VI. But other astronomers, including Romeel Davé of the University of Arizona in Tucson, are skeptical that the Oxygen VI Shull's group has seen resides in WHIM. "The bottom line is that a rock-solid, unambiguous, fully accepted detection of the WHIM has yet to be made," says Davé, adding that next-generation x-ray telescopes could help find the stuff.

Meanwhile, researchers have been improving the baryon inventory through



Thin fog. Artist's conception shows how astronomers have detected warm intergalactic gas by studying its effect on x-ray light from distant quasars.

CREDITS (TOP TO BOTTOM): BENJAMIN OPPENHEIMER; ILLUSTRATION: NASA/CXC/M. WEISS, SPECTRUM: NASA/CXC/UNIV. OF CALIFORNIA, IRVINE/T. FANG ET AL.

other measurements. A team led by Jason Tumlinson of the Space Telescope Science Institute in Baltimore, Maryland, has used the Oxygen VI tracer to make better estimates of the number of baryons floating on the edges of galaxies. Surveying the outskirts of 42 nearby galaxies using the Cosmic Origins Spectrograph on the Hubble Space Telescope, Tumlinson and his colleagues discovered that this circumgalactic medium (CGM) contained almost as much baryonic mass as the stars inside the galaxies did. That helps account for some missing baryons, but CGM makes only a small dent

in the overall missing-baryon problem.

A related mystery is a deficit of baryons in the dark matter halos within which galaxies are nestled. Here, too, astronomers find fewer baryons than expected. The deficit is more severe in small galaxies than in large galaxy clusters, says James Bullock, an astrophysicist at the University of California, Irvine. Davé says that might be because smaller galaxies have less gravitational pull to hold on to their gas when stellar explosions and other violent events hurl it toward intergalactic space.

Accounting for missing baryons on the

cosmic scale as well as in galactic halos should help astronomers understand how cosmic structure and galaxies have evolved. “This low-density material is the basic reservoir for new star formation, and the inflow and outflow of this material from galaxies plays a very important role in how galaxies change,” says Todd Tripp, an astronomer at the University of Massachusetts, Amherst. In other words, looking for missing baryons is not just a bean-counting exercise. It’s a key to understanding how the universe came to be what it is.

—YUDHIJIT BHATTACHARJEE

How Do Stars Explode?

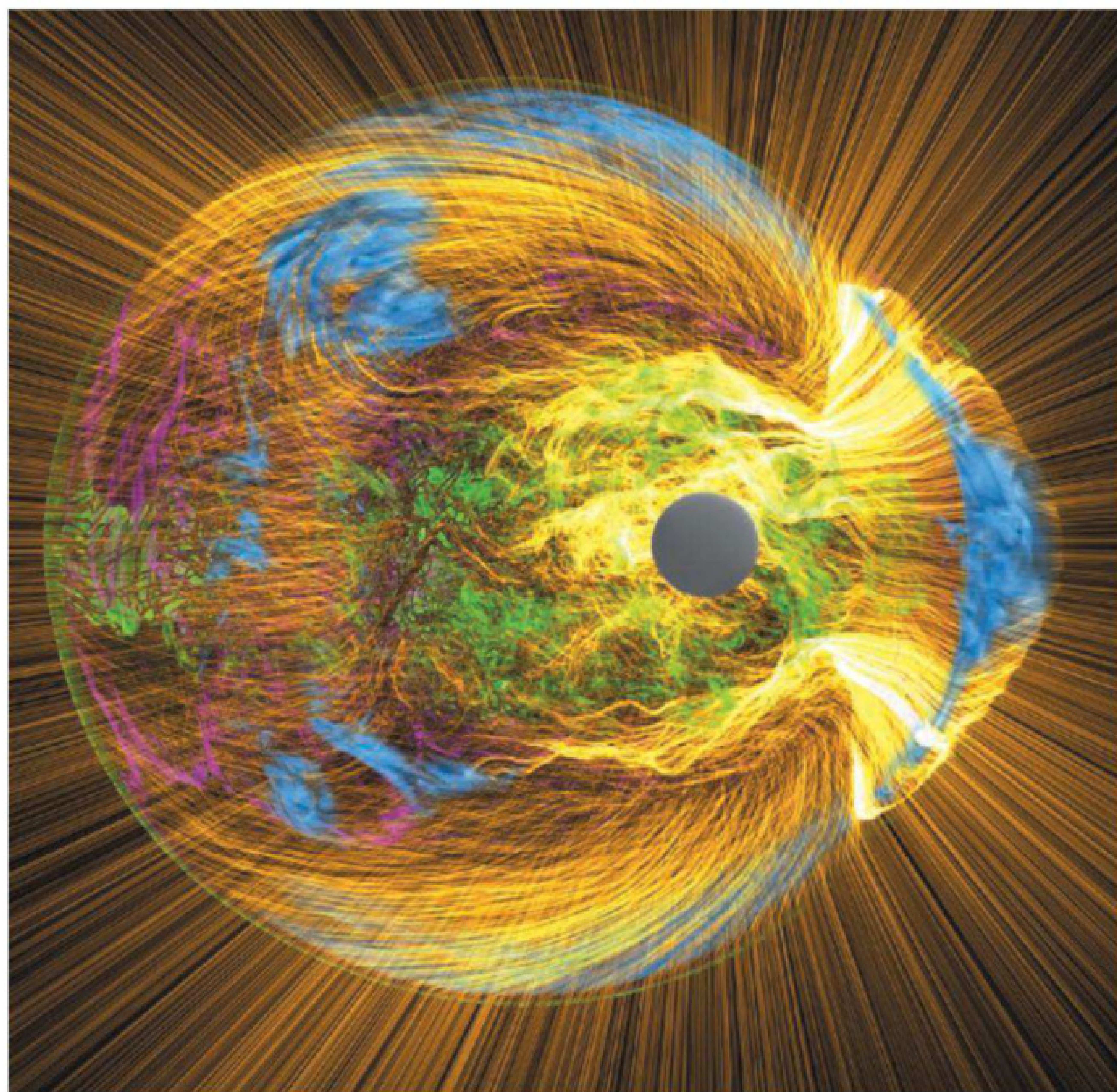
Stars live a glamorous life, shining for millions or even billions of years. For many stars, however, death is far more glamorous. When their fuel has been spent, they explode into a giant fireball known as a supernova, producing the brilliance of multiple suns and sometimes outshining entire galaxies.

How these explosions occur has been a subject of observational and theoretical study for decades. In recent years, advances in supercomputing have enabled astronomers to simulate the internal conditions of stars with increasing sophistication, helping them to better understand the mechanics of stellar explosions. Yet, many details of what goes on inside a star leading up to an explosion, as well as how that explosion unfolds, remain a mystery.

All stars are powered by the same basic process: the fusion of hydrogen into helium, as well as the fusion of light atoms such as hydrogen and helium into progressively heavier elements such as carbon, oxygen, and eventually iron. However, what happens when all the fuel in the stellar core has run out depends on the mass of the star and other factors. That’s why astronomers see different kinds of supernovae across the universe.

One kind, known as a Type II supernova, occurs in stars that are at least eight times as massive as the sun. After such a star has used up the fuel in its core—which turns into iron at this point—it ceases to emit radiation. As a result, the core can no longer generate the radiation pressure necessary to counterbalance the weight of the outer layers. All the material in the core collapses under this weight and gets compacted to form a neutron star. Powerful shocks emanate from the collapse, blasting away the outer layers of stellar material in a fiery explosion.

You might think that bigger stars would



Doomed splendor. Astronomers seek to understand exactly how shock waves from the core of a star blow out its outer shells in a brilliant supernova, as shown in this computer-generated visualization.

make bigger supernovae. But that is not the case: Stars with masses greater than 20 or 25 suns don’t result in type II supernovae. “More massive stars have dense layers of oxygen and silicon just outside their iron cores—and their iron cores are more massive to start with,” says Stanford Woosley, an astrophysicist at the University

of California, Santa Cruz. “As the explosion tries to develop, these dense layers fall in and bottle it up.” Instead of blowing up, the star becomes a black hole.

Another intensely studied category of explosions is type Ia supernovae, which have helped cosmologists measure distances in space and led to the discovery that the expan-

CREDIT: SIMULATION BY JOHN BLONDIN/NCSU. VISUALIZATION BY HONGFENG YU AND KWAN-LIU MA/UNIVERSITY OF CALIFORNIA, DAVIS, AND THE SCIDAC INSTITUTE FOR ULTRASCALE VISUALIZATION

sion of the universe is accelerating. Astronomers think type Ia supernovae occur in binary star systems after nuclear fusion ceases in the core of one of the two stars, turning it into a white dwarf. As the two objects move around each other, gas from the second star starts getting pulled onto the white dwarf until the white dwarf's mass approaches 1.38 solar masses. At that point, the white dwarf collapses and erupts in an explosion of a standard brightness.

Although this general picture of how type Ia supernovae work is widely accepted, many questions remain. For instance, astronomers aren't sure how massive the second star needs to be for the explosion to occur. Astronomers would also like to understand the mechanics of the explosions in greater detail, such as how long it takes for the white dwarf to strip enough material from the other star onto itself, as well as the sequence of events in the final moments before the explosion.

To figure out the blow-by-blow action in these and other supernova explosions, astronomers such as Daniel Kasen of the University of California, Berkeley, have been studying the postexplosion signature of the blasts. "The explosion happens on such a short time scale that typically what is observed is really the aftermath," Kasen says. In recent years, Kasen and others have matched observations with models of this aftermath, detailing aspects that include "what the debris is composed of, how fast the debris is moving, and how light is produced in it," he says.

Astronomers are gaining further insights into supernovae from observations of gamma ray bursts (GRBs): short, intense flashes of gamma radiation emitted by stars. A GRB that lasts 2 seconds or longer is thought to be emitted by a massive, rapidly spinning star as its core collapses into a black hole. A whirling disk of stellar material forms around the black hole, and as this material gets sucked in, two conelike jets of gamma ray particles shoot out perpendicular to the disk.

In several cases, astronomers studying the afterglow of GRBs have been treated to the sight of highly energetic supernova explosions occurring within 2 to 4 days after the burst. As a result, detecting long-duration GRBs has become a "nice trigger for multi-wavelength observations of the coming supernova," says Neil Gehrels, an astrophysicist at NASA's Goddard Space Flight Center in Greenbelt, Maryland, and principal investigator of the Swift orbiting telescope. In recent years, Swift observations have enabled astronomers to study three supernovae in the nearby universe from the very first moments of the explosion.

Even though an analysis of these unusually well documented stellar deaths has helped firm up the link between GRBs and explosions, researchers still don't know precisely how these kinds of supernovae build and explode. The formation of the black hole, followed by the development of the whirling accretion disk that triggers the

emission of gamma ray flashes, appears to be "an evolutionary path that can lead to very powerful supernovae," Gehrels says. In the view of Woosley and others, the rapid rotation of the disk—thought to be responsible for the jets—could be playing a role in the development of the explosion.

—YUDHIJIT BHATTACHARJEE

What Reionized the Universe?

"Cosmologists are often wrong but never in doubt," the Russian physicist Lev Landau once quipped. That might have been true for much of the 20th century, when scientists pondering how the universe worked had many ideas but few data. Over the past couple of decades, however, increasingly precise observations by ground-based telescopes and space probes have shored up cosmologists' standard model, which shows that the universe started in a fiery big bang 13.7 billion years ago and then expanded and cooled. As it did so, tiny initial irregularities in the density of the cosmos attracted matter via gravity to form stars, galaxies, and clusters of galaxies.

But a major gap in our knowledge remains. Some 400,000 years after the big bang, protons and electrons had cooled off enough for their mutual attraction to pull them together into atoms of neutral hydrogen. Suddenly photons, which previously scattered off the electrons, could travel freely

through the universe. A few hundred million years later, something stripped the electrons off the atoms again. This time, however, the expansion of the universe had dispersed the protons and electrons enough so that the new energy source kept them from recombining. The "particle soup" was also dilute enough so that most photons could pass through it unimpeded. As a result, most of the universe's matter turned into the light-transmitting ionized plasma that it remains today.

What caused this cosmic reionization? No one is sure. Astronomers can see the faint cosmic microwave background (CMB), the first radiation liberated when the formation of atoms set light free. Meanwhile, the Hubble Space Telescope and large ground-based observatories have spotted galaxies as far back as about 800 million years after the big bang. Reionization, however, took place between these times, during the "dark ages" in which the first stars and galaxies started to



Way-back machine. The Low Frequency Array in the Netherlands is searching for signs of the neutral hydrogen that vanished from the universe almost 13 billion years ago.

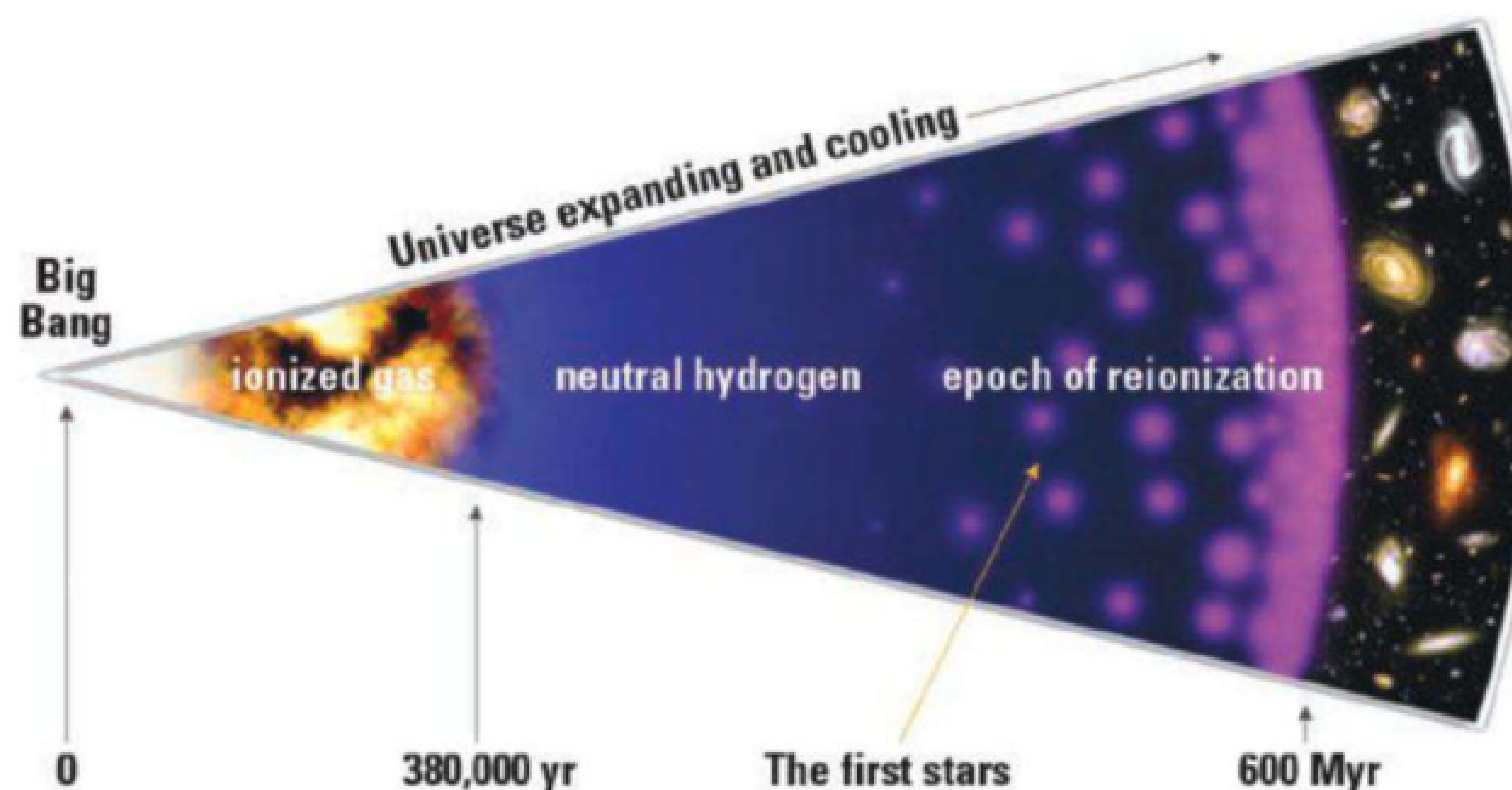
form—a period that astronomers have trouble seeing.

Astrophysicists' best bet is that the energy came from ultraviolet radiation emitted by stars in the first galaxies. In this picture, the galaxies blew bubbles of ionized hydrogen that grew and merged until virtually all of the neutral hydrogen had disappeared. Unfortunately, astronomers—extrapolating from the number of bright, early galaxies Hubble sees far off and the relative abundance of brighter and dimmer galaxies closer to Earth—have worked out that there probably weren't enough galaxies to supply the necessary energy.

But George Becker of the Kavli Institute for Cosmology at the University of Cambridge in the United Kingdom says the model may still be correct. Astronomers may have underestimated either the number of bright stars in early galaxies or the galaxies' porosity to ultraviolet light, he says. Or faint early galaxies may have been more numerous than the population in our part of the universe suggests. Alternatively, other astrophysical objects—such as supermassive black holes or even annihilating particles of dark matter—may have done much of the hydrogen-demolition work.

Answers may come from a larger census of early galaxies and from measurements of their chemical composition and star-formation rates. One or more 20- to 40-meter ground-based telescopes planned for the next decade should see galaxies dating back to when the universe was about 300 million years old, says Richard Ellis, an astronomer at the California Institute of Technology in Pasadena. NASA's \$9 billion infrared James Webb Space Telescope (JWST), due for launch in 2018, should be able to look back about 100 million years earlier still.

Even the JWST, however, has its limits. "There will always be galaxies too faint to see," says Abraham Loeb, a theoretical astrophysicist at Harvard University. Instead of looking for the objects that caused the ionization, Loeb favors what he calls a more "elegant" approach: looking at the hydrogen itself. Neutral hydrogen has a spectral feature that the ionized variety does not: a line at 21 centimeters caused by a flip in the "spin" of the electron relative to the spin of the proton. As the 21-centimeter signal travels through space on its way to Earth, the



Dark times. After particles cooled enough to form atoms, something blew them apart again. Finding the culprit may help researchers reconstruct the history of the first stars.

expanding universe stretches its wavelength to several meters—a phenomenon known as “redshift.” That puts it out of range of traditional radio dishes, so astronomers are building telescopes made of many simple “dipole” antennas spread out across large areas and synchronized by computer. By measuring the strength of the 21-centimeter signal at different redshifts, researchers hope to chart the growth and merger of ionized bubbles around distant galaxies. The information should help identify the ionizing source. For example, because black holes emit x-rays as well as ultraviolet radiation, they would tend to ionize the hydrogen gas more uniformly than some other sources, leading to a less distinct bubble structure.

Among the several 21-centimeter telescopes worldwide, the Low Frequency Array in the Netherlands should start taking data this

process of reionization. Hot gas that cooled relatively slowly, for example, would suggest that the energy for reionization came from energy sources called quasars, which tend to produce more energetic photons than ordinary stars do.

Because cooling changes the way hydrogen absorbs light, astronomers can gauge the temperature of the gas by measuring how it affects the light from more-distant quasars. The technique is less technically demanding than 21-centimeter observations, Becker says. Its main drawback is that it works only for hydrogen that is almost entirely ionized; as a result, the technique can reveal only what happened immediately after reionization, not during the process. “It’s just so difficult to make observations of this early period in cosmic history that you need as many different methods as possible,” Becker adds. —EDWIN CARTLIDGE

Edwin Cartlidge is a science writer in Rome.

winter and might see the line within about 2 years. But detailed observations will probably have to wait for the more powerful \$2 billion Square Kilometre Array, which Australia and South Africa are competing to build over the next 10 years.

One other approach, which might have more to offer in the short term, is to track how hydrogen cooled after heating up in the

What's the Source of the Most Energetic Cosmic Rays?

Fifty years ago in New Mexico, scientists saw a particle that shouldn't exist. A cosmic ray—an atomic nucleus hurtling through space—struck a detector in an array called the Volcano Ranch experiment with an energy of 10^{20} electronvolts, or 100 exaelectronvolts (EeV)—so high that no known process could have produced it.

Nearly 30 years later, at a cosmic ray detector in Utah called Fly's Eye, another one hit. Clocked at 300 EeV, the particle—probably a proton traveling at just 0.000000000000000000005% under the speed of light—packed as much kinetic energy as a baseball hurled at 100 kilometers per hour. Physicists called it the “Oh my

God” particle.

Where had the intruders come from? Astrophysicists were stumped. Fly's Eye gave an approximate direction for the particle it spotted, but no obvious culprit appeared in that part of the sky. The data stream picked up during the 1990s, when the largest cosmic ray detector at the time, the Akeno Giant Air Shower Array (AGASA) near Tokyo, bagged about a dozen more particles with energies of about 200 EeV. The AGASA results galvanized astrophysicists into building a much bigger detector optimized for ultrahigh-energy cosmic rays. In the past few years, the first results from this facility—the Pierre Auger Observatory in western Argentina—

have provided some clues to their origin but, as yet, no smoking gun.

In the 100 years since balloon-borne experiments first showed that a mysterious all-pervading radiation was coming from space, researchers have found out a lot about cosmic rays. They know that 89% of them are simple protons, or hydrogen nuclei. Most of the rest are helium nuclei with a smattering of heavier nuclei, electrons, and antimatter. Astrophysicists think most of the lower-energy particles—up to 10^{10} eV—are from the sun and that those from 10^{10} eV up to 10^{15} or even 10^{18} eV (1 EeV) are from elsewhere in our galaxy. Because cosmic rays with even higher energies seem to come equally from all parts of the sky rather than bunching in the plane of our galaxy, they probably originate outside the Milky Way.

Researchers think midrange cosmic rays get their energy from the shock waves of supernovae. But that mechanism can't explain energies above 10^{15} eV. Theorists have suggested a variety of sources for such ultrahigh-energy monsters: hot spots in energetic radio galaxies, in gamma ray bursts, and in jets streaming from supermassive black holes at the heart of active galactic nuclei (AGNs). Cosmologists have come up with more fanciful ideas, such as the decay of exotic super-heavy elementary particles created in the big bang or the collapse of hypothetical "topological defects" in the universe such as cosmic strings, domain walls, and monopoles.

Whatever their cause, the highest-energy rays probably come from our own neighborhood, cosmically speaking. In the 1960s, theorists calculated that ultrahigh-energy cosmic rays traveling through space lose energy as they interact with photons of the all-pervasive cosmic microwave background radiation. As a result, particles that travel more than about 160 million light-years probably wind up with energy below 50 EeV, a limit called the Greisen-Zatsepin-Kuzmin (GZK) cutoff. In that case, cosmic rays with energies above the cutoff energies must originate in the few thousand galaxies closest to the Milky Way out of hundreds of billions of galaxies in the universe.

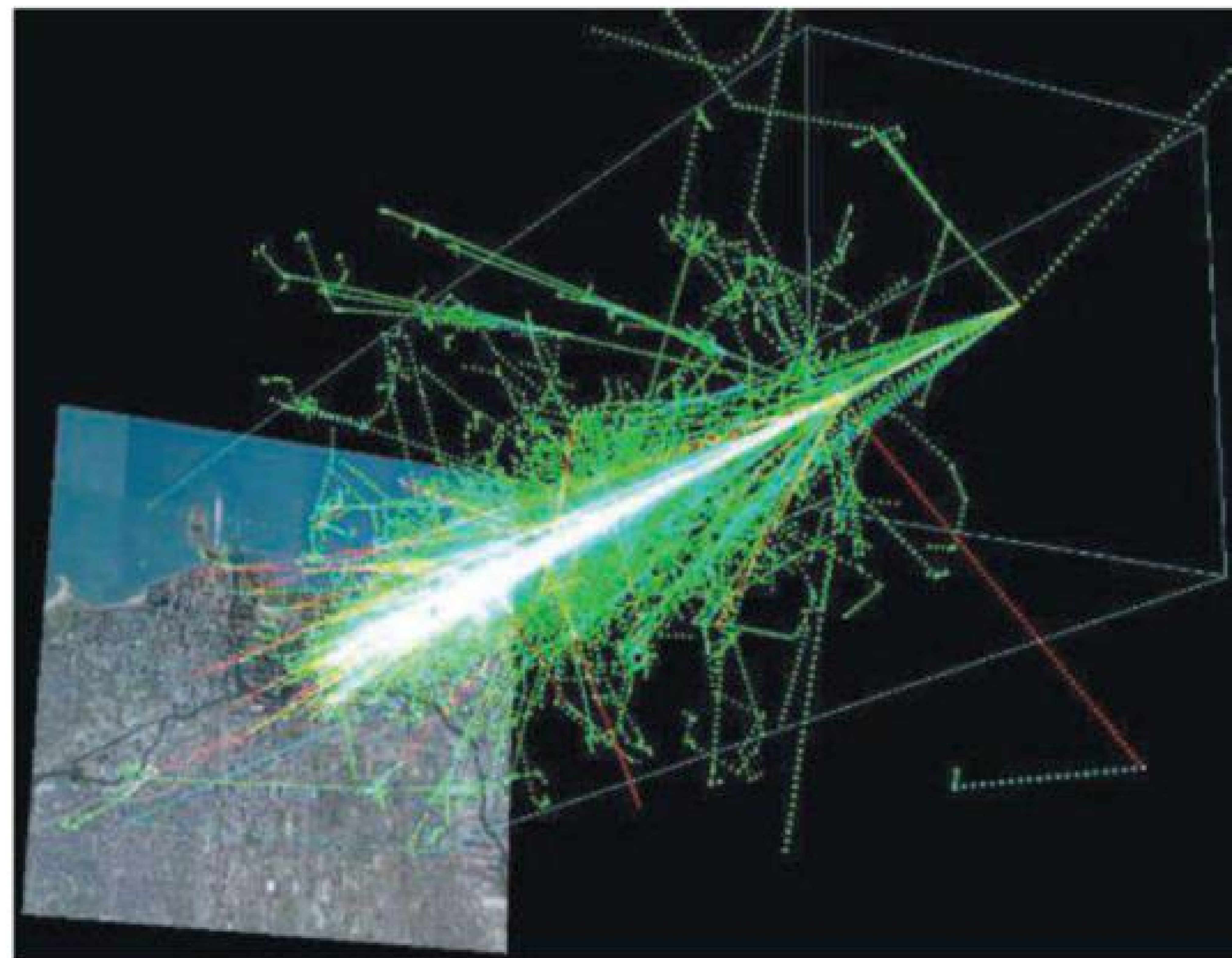
If the GZK cutoff is real, astrophysicists ought to see a sharp drop in the number of cosmic rays at energies above 50 EeV. Do they? For a while, the evidence was contradictory: Between 1990 and 2004, AGASA spotted 11 par-

ticles with energies greater than 100 EeV—more than GZK would suggest—while an upgraded version of Fly's Eye found just two.

The Pierre Auger Observatory aims to settle this dispute and narrow down the list of candidate sources. The observatory consists of four banks of telescopes and 1600 particle detectors spread out over 3000 km² of Argentine pampas. It hunts cosmic rays indirectly by spotting air showers: cascades of other particles that erupt into being when cosmic rays from space plow into the nuclei of atoms in Earth's upper atmosphere. As the path of the air shower follows the track of the incoming cosmic ray, the detectors can reconstruct the ray's original direction.

Auger began taking data in 2005 and 2 years later announced its first result: The GZK cutoff appears to be valid. If AGASA's measurements were correct, the Auger Observatory would have seen about 30 rays above 100 EeV; in fact it saw just two, consistent with the GZK theory.

By 2007, Auger had detected 27 rays with energies greater than 57 EeV and mapped where in the sky they had come from. Although galactic and extragalactic magnetic



Cosmic downpour. A simulation of an air shower caused by a 10^{12} eV proton hitting the atmosphere 20 kilometers above Chicago's lakefront.

fields bend the path of cosmic rays, ultrahigh-energy rays have so much momentum that they follow relatively straight, easy-to-trace paths. The Auger team found that all 27 rays had come from within 3° of an AGN less than 250 million light-years from Earth. The result is suggestive, but the Auger team stresses that it is not proof. At that distance, 3° encompasses so much space that the true source could be something else near the AGN or even in a nearby galaxy. Auger has now observed 113 cosmic rays above 55 EeV; their paths continue to point toward AGNs, although less strongly than the team expected.

Cosmic-ray research suffered a setback in recent years, when shrinking budgets forced scientists to shelve plans for a northern-sky counterpart to the Auger Observatory in the United States. But relief is expected soon in the shape of the Extreme Universe Space Observatory, an orbiting telescope that will look for fluorescent flashes of air showers from above. Designed by the European Space Agency, it was dropped in 2004 only to be picked up again by Japan. The current plan is to launch it in 2016 and attach it to the Japanese laboratory of the International Space Station.

After a century of cosmic-ray research, the most energetic visitors from space remain stubbornly enigmatic and look set on keeping their secrets for years to come.

—DANIEL CLERY



The source? There are many candidates for the origin of ultrahigh-energy cosmic rays, but recent results from the Pierre Auger Observatory point to the jets emanating from active galactic nuclei such as Centaurus A.

Why Is the Solar System So Bizarre?

All manner of planets circling other stars have been popping up of late: big ones, little ones; gassy ones, rocky ones; hot ones, cold ones. But the freakish diversity of worlds starts much closer to home. From the 1960s to the 1980s, space probes returned the first close-up looks at eight of the then-nine planets. To researchers expecting a simple story that would explain what shaped our solar system, the observations sent a sobering message: in your dreams. Today, enigmas such as Mercury's makeup (mostly iron core, with a thin veneer of rock) and Uranus's skewed magnetic field continue to bedevil planetary scientists, and no tidy resolution is in sight.

For a long time, the planetary oddball to beat all oddballs was Pluto. A ball of dirty ice just $\frac{1}{250}$ as massive as the next lightest planet, Mercury, Pluto circles so far from the sun that its year is 248 Earth-years long. Its orbit is oblong, not circular, and is tipped

resemblance of these terrestrial planets ends there. Earth and Venus have approximately the same mass, size, and composition, but Earth is cloaked in a mild, life-sustaining atmosphere while the venusian atmosphere is crushingly dense, acid-laced, and hot enough to melt lead. Earth is an ocean planet; there is no sign Venus ever had an ocean. Earth is encased by wandering tectonic plates bearing continents; Venus has a single, immobile shell of rock. Earth has a magnetic field generated by the churning of its liquid iron core, it has a large moon, and it rotates 365 times per orbit. Venus has neither moon nor magnetic field, and it rotates—backward—less than once per venusian year.

The more diminutive Mercury–Mars pair isn't much better behaved. Mars has twice the mass of Mercury, but its core-generated magnetic field sputtered out early on. Little Mercury, on the other hand, is still churning

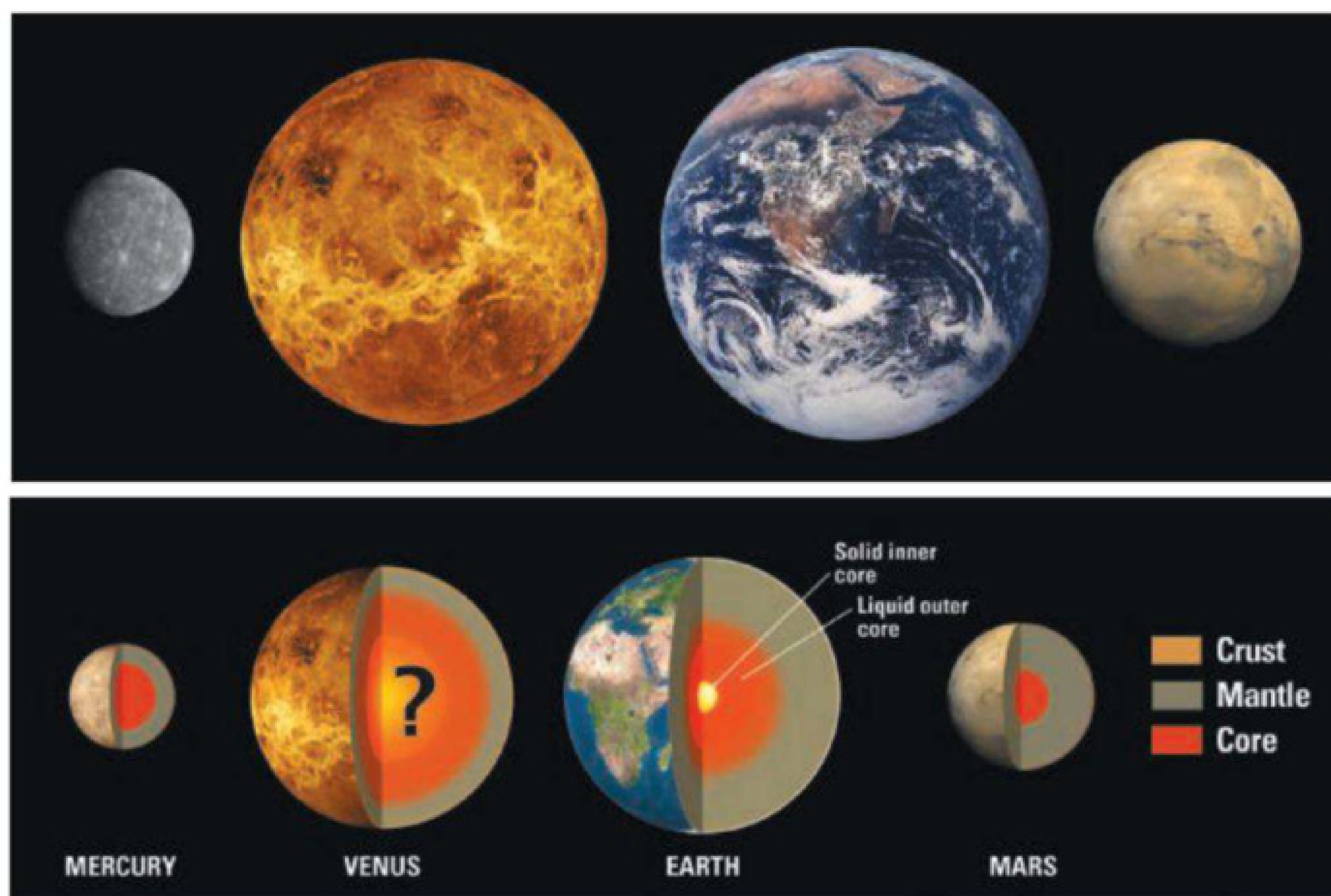
times the mass of Earth but orbit 20 and 30 times as far from the sun, respectively—a region where there was precious little material for planet building when the solar system got its start. Jupiter has a nifty “mini-solar system” of four large satellites, while Saturn has only one large satellite but boasts a set of spectacular rings. And the magnetic fields of the outer planets are mostly awry. Jupiter's is slightly tilted with respect to its axis of rotation—as theory would have it—but Neptune's is tilted 47° and Uranus's a whopping 60° . Saturn's field is perfectly aligned with its rotation axis. That makes for quite the magnetic muddle.

Planetary scientists have some ideas, of course. Orbital distance from the sun was likely a factor in many planetary histories, such as Venus having a “runaway greenhouse.” As for planets that seem out of place, lately planetary dynamicists have been exploring the idea that some may not have always orbited where they do today. Neptune, for example, might have formed closer to the sun, where agglomerating into a planet would have been easier, and then migrated outward.

Looming over all the attempts to explain planetary diversity, however, is the chilling specter of random chance. Computer simulations show that the chaos of caroming planetesimals in our still-forming planetary system could just as easily have led to three or five terrestrial planets instead of four. Mercury may have largely formed with a thick rocky shell only to have it blown away by a chance collision with a still-forming planet nearly its own size. A rare big hit to Uranus might have not only knocked it on its side, where it spins to this day, but also shaken up its rocky core. If so, the more organized churnings of a shallow fluid shell could be generating its magnetic field, producing the observed tilt.

Ferretting out rare random events in the early days of the nascent solar system could be problematic, scientists concede. They may have to settle for working out many of the rules of the planet-making game without pinning down exactly how a particular planetary quirk came to be. Help might come from planets orbiting other stars. As exoplanet hunters get beyond stamp-collecting planets solely by orbit and mass, they will have a far larger number of planetary outcomes to consider, beyond what our local neighborhood can offer. Perhaps patterns will emerge from inchoate diversity.

—RICHARD A. KERR



Family portrait. The rocky planets (from left: Mercury, Venus, Earth, Mars) are a diverse bunch.

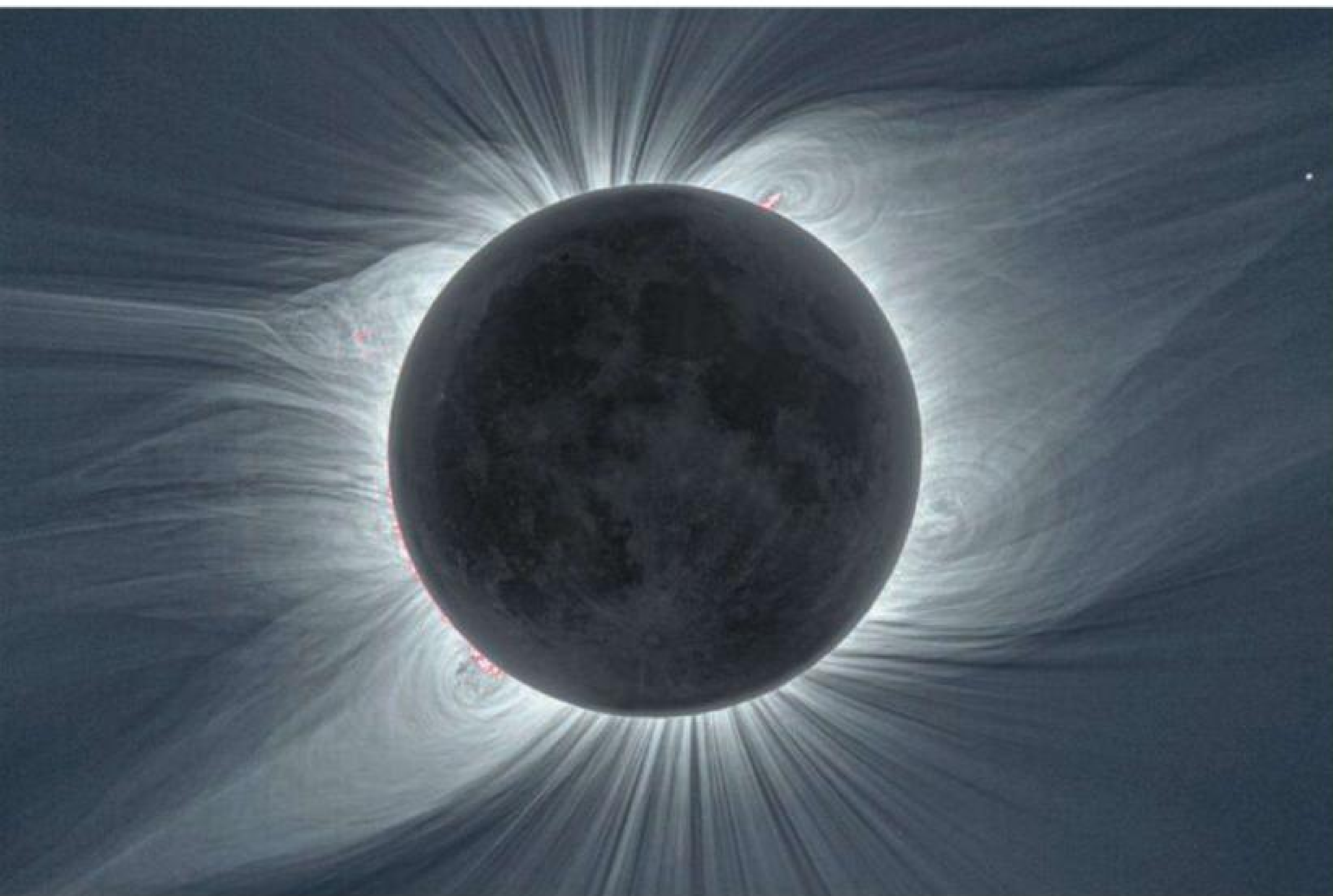
17° to the rest of the solar system. So, many planetary scientists were relieved to realize that Pluto is not a planet at all. The 1992 discovery of other small, icy, airless bodies swarming far beyond Neptune showed that it is just one of the largest of myriad similar objects left over from the formation of the solar system.

The mysteries of the remaining eight planets are proving more recalcitrant. Consider the four innermost planets: Mercury, Venus, Earth, and Mars. All have rocky outer shells and metallic cores, but the family

out a magnetic field, albeit an oddly weak one whose center is far from the center of the planet.

Their interiors differ too. Mars has a proportion of central metal to enclosing rock similar to that of Earth; Mercury is mostly metal. And just last year, researchers learned that Mercury seems to have been made out of rather different primordial stuff than the rest of the terrestrial planets.

The four outermost planets—Jupiter, Saturn, Uranus, and Neptune—have their own quirks. Uranus and Neptune have about 20



In all its glory. The sun's corona reveals itself during a total solar eclipse as the moon passes in front of the 5780 K visible surface of the sun. Here the moon is visible by Earth light. How the sun heats the wispy corona to a million K and more must involve magnetic field lines of the sun.

Solar physicists have made some progress on the heating problem in recent years using observations from the Japanese Hinode spacecraft and NASA's Solar Dynamics Observatory, among others. But much of the wave action suspected of energizing the corona is too quick to be caught and quantified by current instruments. And a nano-flare is still too small to be detected. In fact, solar physicists can't yet directly measure many crucial solar properties above the visible surface, including electric fields, electrical resistance, and the level of wave turbulence; they can only infer these properties imprecisely.

The current fleet of solar spacecraft failed to solve the coronal heating mystery, and no one is promising a solution anytime soon. Still, solar physicists are looking forward to a new crop of solar observatories. NASA's Interface Region Imaging Spectrograph (IRIS) is scheduled for launch this December. The Advanced Technology Solar Telescope is under construction on Haleakalā in Hawaii; its mirror will be more than twice the size of the world's current largest solar telescope. And the European Space Agency's Solar Orbiter, which will be launched in 2017, will get a better view of the solar poles. A common goal of these and other planned projects is to sharpen the view of the sun in both space and time.

A particular target of the coming projects is the chromosphere, the 5000 kilometers or so between the visible surface of the sun and the millions of kilometers of corona. Whatever heats the corona—a little of this, a little of that, perhaps—must pass through the chromosphere.

One key that may be crucial to solving the corona mystery won't involve even glancing at the sun. Three-dimensional simulations of the visible sun—which work much like weather forecasting models—have made considerable strides in recent years. They now spontaneously generate both a corona and a chromosphere, so researchers seeing ever more solar details will also be able to play with their own “sun in a box” to help figure out what makes the corona so crispy.

—RICHARD A. KERR

Why Is the Sun's Corona So Hot?

Yes, the sun is hot—really hot. It's 16 million K hot at its fusion-fueled core, cooling, as the second law of thermodynamics requires, to a still-blistering 5780 K at its visible surface. But for the better part of a century, solar physicists have been mystified by the sun's ability to reheat its corona, the encircling wispy crown of light that emerges from the glare during a total solar eclipse. There, temperatures again soar to 1 million K and more. How would heat dissipating from the core out beyond the surface abruptly punch temperatures up by a factor of 200 and more?

The basics of coronal heating are clear enough. Everyone agrees that there is plenty of energy to do the job in the churning solar interior just beneath the visible surface. And everyone agrees that the sun's magnetic field carries the required energy outward to the corona. But that's where consensus ends. Just how the magnetic field transports the energy is much debated, and how the energy gets deposited once it reaches the corona is even more mysterious. Even so, decades of hard scientific work have narrowed the field of explanations to a half-dozen variants of two main mechanisms.

One popular candidate is heating by magnetic waves. The sun's magnetic field

lines, locked to ions in its churning gases, vibrate endlessly in ways that send all manner of waves and oscillations traveling along the lines. The solar wave of the hour is the Alfvén wave, a twisting oscillation along a magnetic field line. Researchers have studied Alfvén waves in the lab for decades but have only recently detected them rising from the sun; so far, no one knows whether they carry enough energy to heat the whole corona, or—if they do—how that energy could be converted into heat there.

The alternative to wave heating is “nanoflares” produced when magnetic field lines snap and reconnect. The forests of magnetic field lines rooted in the churning visible surface are continually getting tangled, and those tight tangles store a lot of energy. But field lines don't simply untangle themselves to release that energy. Instead, adjacent lines can reconnect, as they do in Earth's magnetic field. Two lines cross, fuse, and then break across the point where they melded to form two hybrid loops. Unleashed, the new loops snap back like slingshots. On the sun, reconnection hurls superheated plasma toward the corona as nanoflares. Whether enough of them occur and deliver enough energy to heat the corona remains unclear.



LETTERS

edited by Jennifer Sills

Reading Too Much Into Baboon Skills?



THE REPORT "ORTHOGRAPHIC processing in baboons (*Papio papio*)" by J. Grainger *et al.* (1) demonstrates that baboons can learn to recognize printed words. The Perspective by M. L. Platt and G. K. Adams ("Monkey see, monkey read," 13 April, p. 168) incorrectly uses these results to suggest that humans could be taught to read by a method that depends on recognition

of the visual letter sequence alone. Moreover, the Perspective suggests, contrary to the evidence, that dyslexia could be remediated by a similar visual method.

A reader's task is not to distinguish the large class of real words from nonwords (as the baboons did) but to connect a specific printed word with the corresponding word in his/her spoken language. In addition to recognizing words seen before, the reader must reliably iden-

tify words that have not previously been encountered in print. There is a big gap between the skill of learning visual redundancy and the skill of recovering the spoken equivalent of a new printed word; the experiment of Grainger *et al.* does not inform us about the second process.

Grainger *et al.* cite neurobiological evidence that skilled readers use a cortical visual pathway to recognize words. Not mentioned, however, is evidence that the path to becoming a skilled reader involves developing the cortical substrate necessary to recognize letter-sound relationships before developing the fast-mapping visual circuit (2, 3). The implication that learning to read can be successful without acquiring the letter-sound relationship runs counter to the great bulk of scientific evidence collected over the past few decades (4, 5).

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Response

WE AGREE WITH KATZ *ET AL.* THAT READING is more than orthographic processing. True reading requires a mapping of visual forms onto arbitrary symbols to give words meaning. Notwithstanding our tongue-in-cheek title ("Monkey see, monkey read"), baboons clearly cannot read. Nor are they likely to ever acquire that skill, for precisely the reasons Katz *et al.* outline. Nevertheless, several lines of evidence, including the work by Grainger *et al.* (1), suggest that reading is not yoked ineluctably to spoken language. Foremost, many individuals—notably the congenitally deaf—learn to read without ever hearing speech.

Grainger *et al.* only examined orthographic processing, a skill that is necessary but not sufficient for reading. Their findings suggest that the formation of letter-sound rela-

tionships is not necessary for orthographic processing but address neither whether such a skill is necessary for reading generally, nor whether it plays a facilitative role in learning orthography in humans. Although Grainger *et al.*'s finding—that baboons can learn the statistics of letter co-occurrence inherent in English—has strong implications for understanding the neurobiological mechanisms supporting reading and their evolution, this is distinct from the question of what methods will be most successful in reading education. We acknowledge the substantial literature supporting the importance of phonological coding in learning to read.

In our Perspective, we made no specific recommendations for teaching children to read or remediating dyslexia. Nevertheless, understanding the neurobiology of reading may suggest new ways to remediate dys-

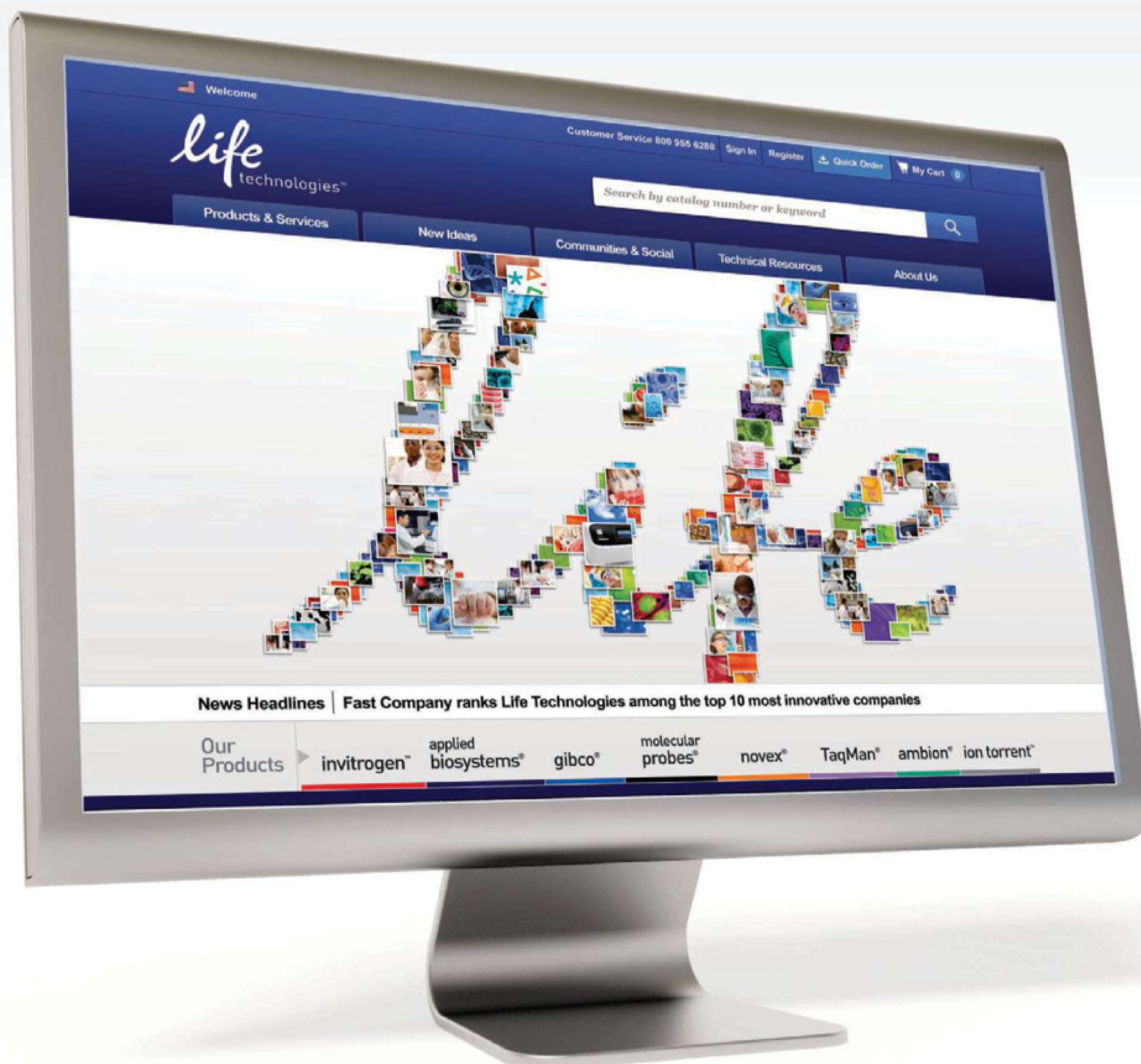
lexia. Recent work has emphasized that different subtypes of dyslexia, characterized by specific functional deficits, reflect dysfunctions in distinct neural circuits, the relative importance of which can vary with both the individual and the writing system (2, 3). The presence of dyslexia in deaf individuals further demonstrates the need for an increased understanding of the various neural systems that can support reading (4, 5), given that the reading strategies employed by some deaf readers may differ from those used by individuals with intact hearing (6, 7). If the etiology of some cases of dyslexia involves specific deficits to orthographic processing, and orthographic processing relies on conserved neural systems for processing visual form and objects, then interventions targeted at these visual deficits may benefit these individuals.

MICHAEL L. PLATT* AND GEOFFREY K. ADAMS

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Going to Bat for an Endangered Species

THE LAST SURVIVING (1) OF THE THREE Mascarene-endemic fruit bat species of Mauritius (*Pteropus niger*) now faces elevated extinction risks, as the government disregards science and bows instead to the demands of industry. In response to pressure from fruit growers, the Mauritian government is working to amend the country's law that protects bats (2). Meanwhile, the Parent Ministry of the National Parks and Conservation Service is calling on the World Conservation Union (IUCN) to review the bat's threat category (3, 4). Together, these moves will enable culling of an endangered species (2). Conservationists' appeals to the government to adopt a more evidence-based approach have gone unheeded.

In 2008, the species' IUCN Red List category was reclassified from Vulnerable to Endangered (5). The bats have suffered extensive habitat loss and degradation (6), and they are highly vulnerable to stochastic events like cyclones (1). Legalizing culling would add to these pressures, putting the species at further risk. The government's move is particularly troubling because it coincides with a recent policy that is restrictive to conservation research and local capacity building in conservation (7).

Seeking to cull a species with a recent history of worsening conservation status will be detrimental to Mauritius's good reputation (1, 8), built on having saved several endemic species from extinction (e.g., the pink pigeon, *Columba mayeri*) (1). The government has lost further credibility by providing no reasonable evaluation of expected benefits of specific quotas of bat culling. Moreover, the current bat protection law has proved difficult to enforce (2, 5), which casts serious doubts on the government's ability to enforce culling quotas in the future.

The international community should encourage Mauritius to conserve the bat pop-

ulation by exploring and extending alternative programs, such as protective netting. Mauritius should not undermine the bats' key ecological role as the largest surviving frugivore in the island's threatened native forests (9).

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Biosecurity on Thin Ice in Antarctica

SINCE THE ESTABLISHMENT OF THE PROTOCOL on Environmental Protection to the Antarctic Treaty (Madrid Protocol) in 1991, visitor numbers to the continent have increased almost 10-fold (1), bringing with them alien marine invertebrates (2), terrestrial weeds (3), and animal diseases (4). The International Association of Antarctica Tour Operators (IAATO) encourages tourists not to "pack a pest" (5, 6), but recently published evidence from a continent-wide survey highlights that this is insufficient to prevent tourists from introducing alien plants (7). Worryingly, this survey, which was undertaken as part of the International Polar Year (IPY), reveals that, on a per capita basis, it is scientific personnel

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LETTERS

rather than tourists who pose the highest risk of introducing alien species on their clothes and equipment.

Whose responsibility is it to prevent or manage alien invasive species in Antarctica? The Madrid Protocol prohibits the introduction of any alien species in an effort to minimize their interference with native wildlife, but all evidence to date points to a failure in its implementation across the continent (8). Sufficient data now exist for signatories to implement robust biosecurity policies on sea and land, initiate alien species management programs, audit new research initiatives, and monitor compliance of both public and private operators. Australia and New Zealand are world leaders in managing invasive species (9), and their territorial claims in the region put these countries in a unique position to lead a coordinated biosecurity strategy across the entire continent. The establishment of an International Antarctic Biosecurity Agency, funded through subscriptions from signatories to the Antarctic Treaty and a visitor levy, would be an essential first step to deliver on the full aspirations of the Madrid Protocol. The Antarctic Treaty System is often cited as a flagship example illustrating that the international community can satisfactorily govern the commons. It is time to put this into practice by moving beyond documenting biological invasions and toward managing the problem. The IPY data provide the first continent-wide evidence that knowledge of impacts has not kept pace with the high rates of species introductions. The difficulty in eradicating established invasive alien species in these pristine environments demands a greater emphasis on risk prevention rather than minimization (10) to be urgently adopted in existing management plans.

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CORRECTIONS AND CLARIFICATIONS

Letters: "Presumed guilt in the anthrax case" by J. Guillemin and "Response" by D. A. Relman (11 May, p. 669). Due to an editorial error, an incorrect version of the text was published. The original author names, affiliations, and references were correct. The text has been corrected in the HTML and PDF versions online. The correct text follows.

Letter: In his review of my book *American Anthrax* on the 2001 anthrax letter attacks ("Have we 'met the enemy?,'" 3 February, p. 540), D. A. Relman accuses me of imposing a "presumption of guilt" on the FBI's prime suspect, U.S. Army microbiologist Bruce Ivins. In fact, I described many ambiguities in the case against Ivins and took no personal position on his guilt or innocence. I relied on the report of the National Research Council (NRC) committee that evaluated the FBI science, and, regarding a possible foreign source for the letter spores, I accepted its conclusion that "We consider these data to be inconclusive regarding the possible presence of *B. anthracis* Ames at this undisclosed overseas site" (1). Relman served as vice chair of the committee that reached this conclusion.

Response: In my review of *American Anthrax*, I sought to highlight the valuable contributions of this book to the public understanding of this complex and controversial case, as well as the book's shortcomings. It is true that Guillemin makes no explicit statement about her position regarding the guilt or innocence of Ivins. However, statements such as, "the FBI had solid scientific proof that the spores in the anthrax letters matched those in a flask, labeled RMR (Reference Material Receipt)-1029, that was in Ivins' keeping at the Army's medical institute at Detrick" (p. xxii), and "a criminal—either Ivins or someone else—had used the institute's Ames anthrax spores to commit murder" (p. 251), misrepresent the strength of the scientific evidence that points to this flask as the source of the spores in the letters (and by inference, Ivins and/or the Army's institute at Fort Detrick, MD). A variety of weaknesses in the FBI's scientific investigation are discussed in detail in the NRC Report (1), but unfortunately, these weaknesses are not given much coverage in her book. Guillemin is correct: I served as vice chair of the NRC committee that issued this report.

Letters: "Conservation concerns in the deep" by A. C. Hartmann and L. A. Levin (11 May, p. 668). Corresponding author A. C. Hartmann can be reached at achartma@ucsd.edu.

Letters to the Editor

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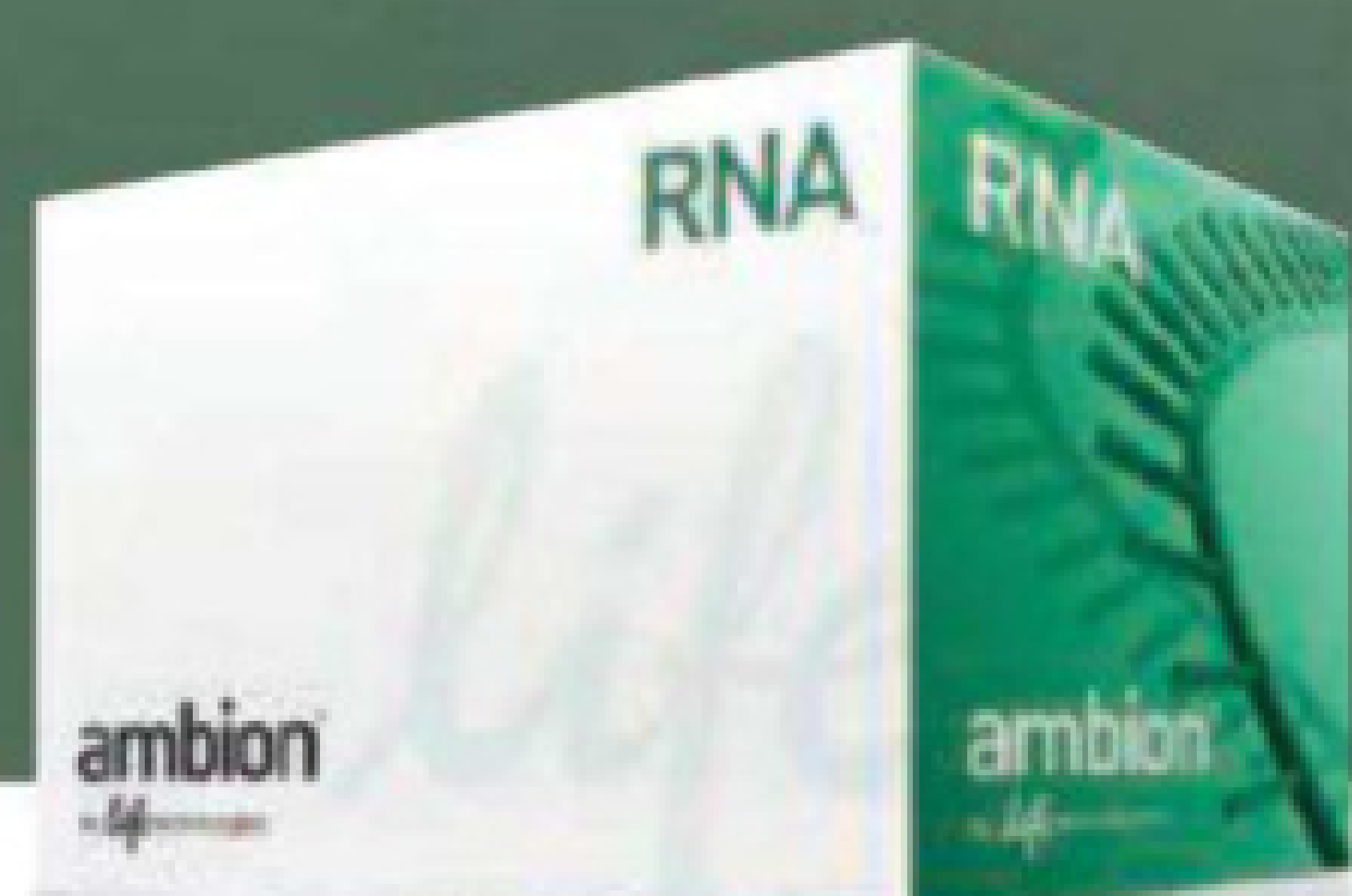
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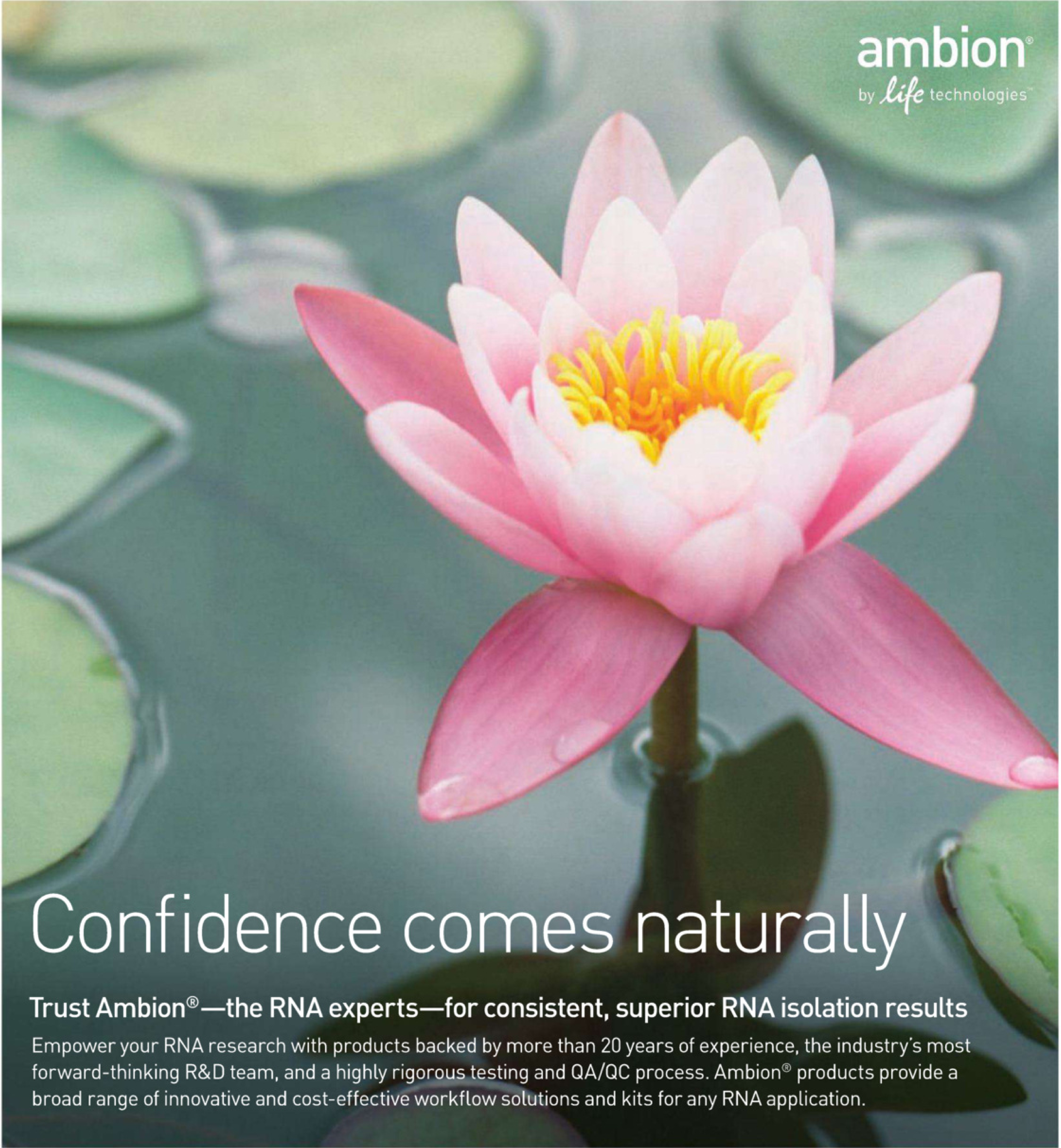
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COMPUTER SCIENCE

To Flourish in the Online World

James A. Hendler

I remember a time back in the 1990s when a group of my friends were discussing *Men Are from Mars, Women Are from Venus* (1) over dinner. Everyone agreed that the book had some parts that were obvious and others that were surprising. As the discussion went on, we increasingly realized that which parts were which was not something everyone agreed about—roughly speaking, the men and the women found very different parts helpful in understanding each other's lives.

I was reminded of this as I was reading Howard Rheingold's *Net Smart*. I felt the same sort of mix of "I knew that" and "really?" I realized that some aspects of the book would be more surprising to "digital natives," who have grown up in the presence of the Web, and others would be more useful to "digital aliens," including those scientists trying to figure out what students are doing on Twitter or wondering whether to join Facebook. Web-savvy readers may be surprised to learn some of the tricks Rheingold offers for increasing one's Web awareness, while others, still working out what the Web can offer them in doing their jobs, will appreciate the insights he proffers as to making better use of the electronic world.

What's so special about this book? Back in 2006, I was one of the authors of an article arguing for the need for a more systematic, interdisciplinary, scientific approach to the study of the World Wide Web (2). Since then, the field increasingly known as Web science has grown to include an international conference, new educational programs (from undergraduate to Ph.D.) around the world, and more and more labs devoted, at least in part, to the topic. However, as with any emerging discipline, the research still tends to be diverse, common methodologies have yet to be defined, and developments have been hard to track. The first serious popular science account of Web science, *Net Smart* provides an overview of much of the field's best work.

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Net Smart

How to Thrive Online

by Howard Rheingold

MIT Press, Cambridge, MA,
2012. 332 pp. \$24.95.
ISBN 9780262017459.

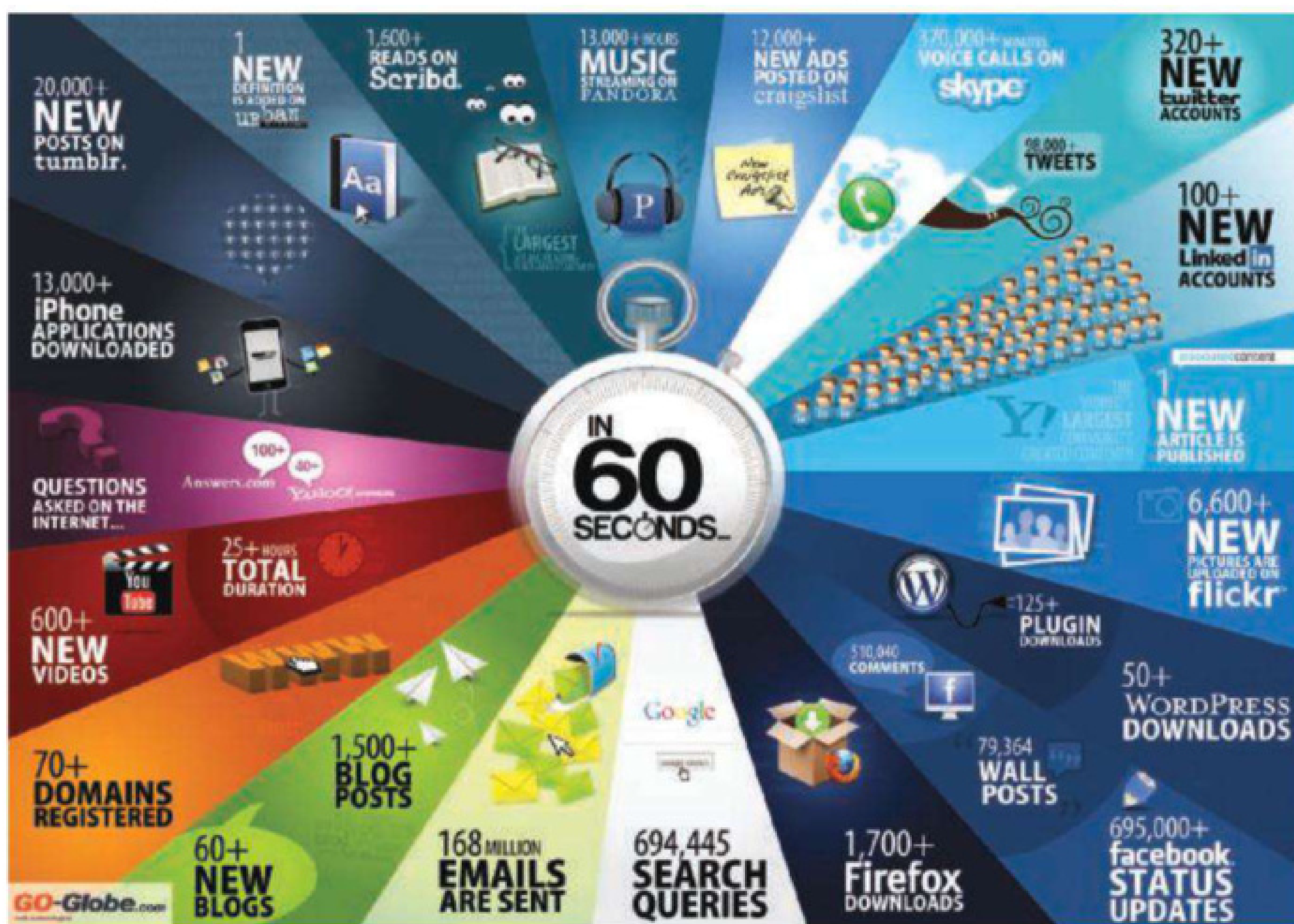
That Rheingold has written a smart and enjoyable guide is unsurprising. An Internet user from before the Web, he was involved in the creation of the Whole Earth Catalog and was a founder of *HotWired*, one of the first successful online content magazines. Through earlier books (3, 4), he helped define and explain emerging Internet trends. [To learn more about Rheingold, I recommend Wikipedia (5); for more about Wikipedia, turn to his chapter "Social-digital know-how," which includes excellent coverage of collective intelligence.]

Rheingold addresses the need to be smarter about our use of networked technologies arising from the tensions between the Web as a distraction and as an essential part of life. As he puts it, "Since the early 1980s, however, the digital world along with the network of fascinating but potentially distracting links, videos, tweets, emails, wall posts, and wittily captioned photographs of cute cats has been both the seductive distraction and relevant knowledge flow that have competed for my mind." He argues that being able to function efficiently in this welter of information requires five "literacies": attention,

crap detection, participation, collaboration, and network smarts. In successive chapters, he provides definitions of these literacies, reviews the research most relevant to them, and produces strategies that can be put to use in one's day-to-day online life. Rheingold writes in the first person, discussing his own encounters with both the technologies and the researchers behind the strategies he offers.

The book is not without its flaws. The chapter on attention reviews some of the best research but is not as prescriptive as some of the others. Rheingold is a practitioner of yoga and meditation. While I'm willing to believe that this helps him, I'm not convinced that better breathing is the best way to deal with the attentional demands of online life. The discussion of crowd-sourcing suffers from a bit of Silicon Valley myopia, missing, for example, some of the very different ways that Oriental communities come together in what the West has, somewhat incorrectly, called "human flesh search." And I'd be remiss if I didn't point out that the diagram labeled "small world network" doesn't show one.

Despite these and other small problems, I really liked *Net Smart*. The author's recounting of his online life and strategies rings true, his review of the literature in the field includes much of the best work in the area, and—despite being a professional Web scientist and a Webby for over two decades—I discovered some great advice and pointers to applications I wasn't aware of (which have already started to bear fruit in my own online work). The Internet has changed how we do science today. Whether teaching in the class-



Every 60 seconds on the Internet.

room, doing research in our laboratories, or communicating with our colleagues around the globe, we cannot ignore the growing demands and distractions of the Web. Rheingold does us an important service by offering a number of insights into, and strategies for, the “net smarts” we need to function more efficiently in our increasingly online world.

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10.1126/science.1221298

ENVIRONMENT

Sharing Wonder in Nature

Kirsten Rowell

Sharing knowledge and wonder about nature with our children has for millennia been part of who we are as humans. Leading our children in hand and following them on muddied knees, we have explored nature around us—playing, working, gathering, and passing down our observations and insights into how we perceive the world. It can be argued that our capacity to explore and wonder about the natural world underlies our proclivity for collecting observations and communicating ecological knowledge and gives us tools to adapt to new landscapes, build communities, and advance civilization. But as Rachel Carson noticed over a half century ago in her essay “Help your child to wonder” (1), engaging children in nature-focused activities “was hardly a conventional way to entertain one so young.” And the situation is much worse today, when contemporary culture has lost the practice of spending substantial time in nature with children (2).

Inspired by Carson’s essay, Julie Dunlap (the author of several children’s books about nature) and Stephen Kellert (a social ecologist at Yale University) have collected in



Nikki McClure’s papercut *Rely*.

Companions in Wonder a set of contemporary essays on childhood and nature. Their anthology provides a diverse set of often provocative examples that illuminate the importance of exploring nature with children. The essays initiate a dialogue beyond the children-in-nature and environmental movements, and the collection reaches out to a wide audience by including voices and perspectives not often heard in discussions of the place of nature in children’s lives.

Dunlap and Kellert gathered 31 “considered reflections on adult-child relationships outdoors.” The writers’ voices are strong, and the experiences they describe are deep and visceral. As a body of work, the essays stitch together a leafy, rich canopy of answers to the question “Why do we need to share and explore the natural world with children?” The contributors include some icons of recent nature writing—such as Barry Lopez, Alison Hawthorne Deming, and Kathleen Dean Moore—but also less well-known writers. All of these authors have thought deeply about how childhood experiences influence adult relationships with and attitudes toward nature.

The book as a whole crosses generations, cultures, and religions to explore our links with nature. Susan Cohen, Stephen Lyons, and Scott Russell Sanders share personal stories of how nature can be a catalyst for healing relationships and recovering from life’s traumas. Janisse Ray speaks to the exhausting work of raising a child alone, making difficult life-style decisions while doing the guessing work about what you want for your child and what they need for themselves. Michael Branch uses humility and humor to

expose the obsessive zeal that can leak into even the most well-meaning endeavors, exploring his own crazed attempts to build a teaching garden in the high deserts of the Sierra Nevada for his four-year-old daughter. Such stories provide concrete examples of how engaging children in nature can be seen as a civic duty—fostering love, imagination, confidence, and, in the end, a sense of stewardship for land and water.

This volume also reveals both systemic weaknesses and growing opportunities for building a stronger and more resilient nature movement that will engage children and adults (together) in the

natural world. The selections dig deeper than the traditional ideals that are bedrock to the nature movement, and the topics touched reach beyond the typical affirmative prose that one would expect from the book’s title. The writings by Danyelle O’Hara and Laurel Savoy extend to the roots of why environmental movements are blind to their own racism. Contributions by David Mas Masumoto, Margo Tamez, and Carolyn Finney demonstrate how time in nature has rewarded cultures very differently, showing us through personal childhood experiences how land, labor, class, and race all come together to color our relationships with the natural world

and with each other. By highlighting that our relationships with nature are nestled within historical and social contexts, these stories expose an often-unstated truth: not everyone experiences nature with the same emotion. The diverse perspectives and emotional bonds brought to light through the range of stories told in

Companions in Wonder need to be reconciled before we can heal our broken relationships with nature. We must acknowledge that our relationships with nature are not always idyllic or even benign if we are to build a children-in-nature movement that reflects the diversity of who we are, how we came to be, and why we care about our children and the natural world.

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MEDICINE

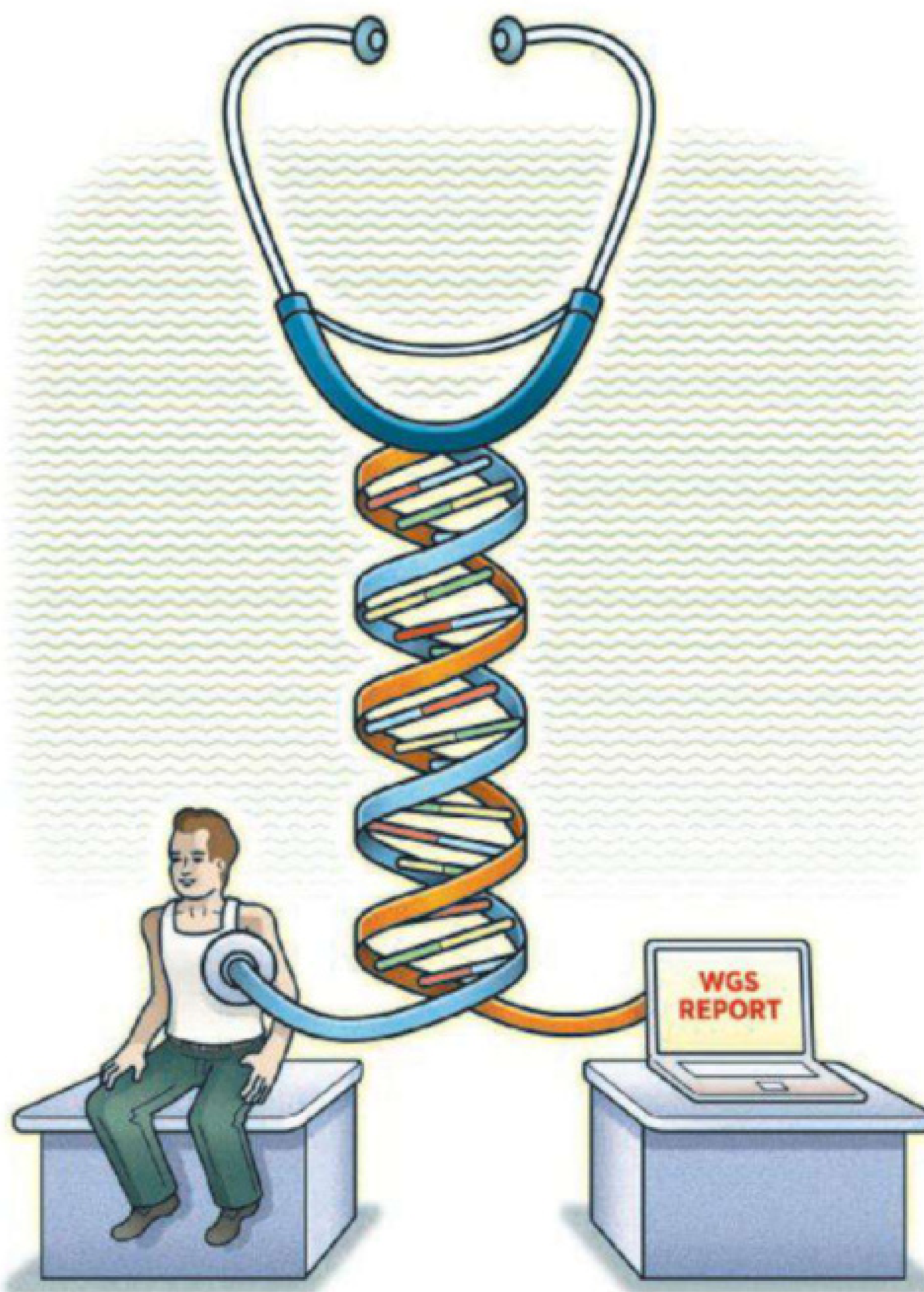
The Ultimate Genetic Test

Radoje Drmanac

Encoded in the DNA sequence of our individual genome is the genetic program to build, maintain, and adapt all our tissues and their functions. Each human genome contains 200,000 coding elements (exons) and millions of regulatory elements (1) defining complex signaling and regulatory networks.

Compared with the reference sequence generated by the Human Genome Project, any single individual's genome has about four million sequence variations. Although most of these variants are harmless, some cause disease, predispose people to conditions (2), and determine responses to treatments. In general, the impact of genetic variants is disproportionate to their frequency. For example, some genetic diseases in newborns are frequently caused by de novo personal variants, which include large DNA deletions or duplications (3–5).

Today, a limited number of variants are detected through targeted genetic tests including some newborn screening tests. Some recent sequencing-based tests target a few hundred genes (6). Whole-genome sequencing (WGS) is already a powerful research tool to study the molecular basis of thousands of diseases. In addition, doctors from at least a dozen large, especially university-related, hospitals also use WGS for some of their patients (mostly with idiopathic diseases or refractory cancers) (7–9) but usually as part of a clinical study because only a few laboratories generate WGS in certified (CLIA) labs. In some cases, these labs have recently performed sequencing of all of the exons and, if unable to find a genetic cause, have turned to WGS. A few doctors have “prescribed” CLIA-performed WGS to better understand a condition or as supporting information to potentially prevent disease or improve health (10). A more effective approach might be to routinely sequence



individuals' entire genome once, preferably early in life, and to continue to use this information to make health-care decisions throughout their lifetime.

The Comprehensive Genetic Test

Similar diseases can result from many different genetic changes, including rare family variants and de novo mutations, in one or multiple genes and regulatory regions (11) that affect a molecular pathway. However, a single genetic variant may have a very different impact on health depending on the other genetic variants that exist in the genome (e.g., modifier genes), environmental factors, or a combination of both. This is why many genetic tests targeted to specific parts of genes have limited explanatory value. For example, it is now accepted that complete sequencing of *BRCA1* and *BRCA2* genes is more informative than targeting only the exons and nearby splicing regions. Sequencing additional tumor suppressor genes may

Individual whole-genome sequencing has the potential to greatly improve disease prevention, diagnosis, and treatment.

provide even more powerful information about the basis and progression of cancer (12). By extension, sequencing an individual's entire genome and identifying the variants in all coding and regulatory regions is perceived to be critical to untangling complex regulatory interdependencies in cancer and other diseases and to potentially help with disease prevention, diagnosis, and treatment.

Use of WGS would reduce the undiagnosed cases that result from rare or de novo variants in any of thousands of genes. For example, standard tests for cystic fibrosis miss 20% of carriers in some populations (13). Because 50% of individuals with *BRCA* mutations have no familial association (12), in the absence of family history, a doctor may not even recognize a need for a targeted test. Accurate WGS would also detect adverse reaction to drugs (14) or anesthesia (15) due to various rare vari-

ants. Similarly, this may also be used to help ensure healthy offspring via carrier, preimplantation, or prenatal screens. Furthermore, the complete sequence enables more informative and accurate contextual interpretation of detected genetic variants for presymptomatic predispositions to diseases and help with preventive treatments.

The availability of an individual's WGS would provide immediate access to genetic information and its latest interpretation when needed, e.g., the response to clopidogrel as an urgent heart treatment (16). This readiness would maximize disease diagnosis, prevention, and treatment, such as sudden cardiac arrest, while avoiding delays due to serial hypothesis testing.

Instead of handling different types of partial data sets or making decisions without genetic data, once comprehensive and ultimate genetic information provided by WGS is broadly accepted, many health-related industries can standardize their approaches

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around WGS data. This would eliminate the need for development of targeted genetic tests or companion diagnostics for each of the hundreds of novel drugs. It would facilitate more informative and efficient disease studies, drug development, and clinical trials. WGS should also lower health-care costs as the result of disease prevention and reduced use of many diagnostic techniques and untar-geted treatments.

As with other genetic tests, genome sequencing is noninvasive, as it usually requires only a small sample of saliva or blood. After sequencing is completed and the variant data with supporting statistics are stored, an individual's inherited genome can be queried to help answer health questions over a person's lifetime. A computer program could issue an up-to-date report that lists any actionable warnings or recommended actions. These ever-improving analyses and reports could be tailored according to a person's age, prescription drug use, and reproductive or other choices. Many WGS findings might be automatically reported only later in life when they become actionable. In addition, the person's doctor could initiate other disease-, gene-, or pathway-focused queries to address specific medical issues.

WGS is even more informative and more accurate when included as part of a family genomics program in which all members of a family have their genome sequenced (7, 10). Ultimately, the main goal of WGS and genomic medicine is not only to measure personal propensity to a disease but also to determine genome-specific ways of preventing diseases and improving treatments. The overall clinical value is difficult to define today, as WGS has not yet been used over the course of any one life-span, let alone many, full life-spans. However, values for specific applications will soon be measured as thousands of WGS tests are performed. There is a growing number of examples of patients who have already been molecularly diagnosed (7, 17) or successfully treated (8, 18) after the genetic causes of their disease or condition have been discovered by WGS.

Technical Progress and Challenges

Sequencing and analysis of the human genome's six billion base pairs requires extreme accuracy to prevent errors. Current WGS technology, in spite of accuracy of only one false single-nucleotide variation per 500 kbp (19), requires improvements for broad use in clinical applications. Because the human genome is diploid, it is also critical to determine which variants come from which parent (20) in a cost-effective and scal-

able manner (21). Currently, long haplotypes and some repeat regions are missing, but that and variant calling accuracy is likely to substantially improve in a few years. In the meantime, actionable WGS-detected variants can be validated by confirmatory testing.

To achieve a broad health impact, millions of human genomes will need to be sequenced each year. With current projected improvements in sequencing instruments (22), a rate of over one million WGS tests per year will be reached in this decade. Also, more CLIA-certified WGS labs are coming online. Furthermore, there are continuous cost-efficiency improvements in WGS (23, 24) and, although not achieved yet, for a genome sequence to cost less than \$1000 seems a reachable goal (22). Clinical use of WGS will have additional costs related to operation of certified labs; the need to guarantee a higher accuracy (e.g., the need for higher read coverage or variant validation); and the cost of life-long data storage and clinical (re)interpretation.

Genomic knowledge is growing exponentially, fueled by large-scale sequencing projects such as Encode or the Personal Genome Project (25), the thousands of whole human genomes being sequenced around the world (>10,000 completed since 2009 and 30,000 expected this year); the increasingly common attachment of phenotypic information to genotypes; and fast improvements in genome interpretation software.

Concerns and Policies

WGS as an ultimate genetic test could be ordered for many indications (8, 17, 18) or as a "screening" test (10, 26, 27) for disease prevention and all other uses. Thus, it is important to begin developing policies and recommendations to facilitate clinical adoption of WGS (28). Regulatory agencies and leading professional societies must work together to provide technical and interpretive standards and guidelines for WGS to assure sufficient quality and usability of the data needed for various applications. Industry standards for the format and content of genomic variant lists and interpretation reports are also required. Reliable specificity and sensitivity statistics should be established for various types of variants and distinct genomic regions. The medical usefulness of diverse genomic elements needs to be established through translational research.

There are many other questions to consider. Should WGS results be stored as part of medical records? How will patient autonomy and privacy be protected? If WGS is done early in life, who decides which data can be released until a child is old enough

to decide? Who pays for WGS, and, if paid directly by patients, under which conditions can they order it directly? How can WGS be made affordable for everybody? It is also important to guard against the overpromise of WGS benefits and to work to prevent unethical and illegal use of our WGS data by others. Thus far, the U.S. Genetic Information Non-discrimination Act of 2008 (GINA) regulates such use in part, but many more public policy questions will need to be addressed by regulatory or legislative action. Both physicians and the public need to be educated about the benefits and limitations of WGS, as patients will play a vital role in WGS adoption.

By its nature, WGS provides foundational medical information which is expected to play a critical role in health management and in improving doctors' decisions in diagnosis and treatment. Today, though, it is important to follow the "first do no harm" maxim. This means constraining WGS reporting to prevent overinterpretation due to limited understanding of the genome (29) but also using WGS instead of less-informative targeted tests whenever feasible. For now, we should treat most WGS reports as indications requiring further investigation and explain this clearly to users who receive this information. In many cases the complete knowledge of inherited genomic variants alone is not sufficient, because it does not take into account the genome-specific impact of environmental and stochastic factors such as noninherited cancer-causing mutations.

The availability of WGS provokes many important social and ethical questions, such as informed consent and access to one's own genetic information. In continuing this debate, we should cherish our personal genome diversity and celebrate the advances in technology and science that are enabling broader adoption of WGS and that may help people live healthier lives.

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MEDICINE

Whole-Genome Sequencing: The New Standard of Care?

Liam R. Brunham¹ and Michael R. Hayden^{2,3*}

Rapid advances in DNA sequencing technology have made whole-genome sequencing (WGS) both technically and economically feasible. WGS has been used with great effect in specific settings to clarify molecular diagnosis and even to guide therapy (1, 2). But are we ready for the routine use of WGS in the care of healthy individuals?

The Problem of Overselling

Much promise has been ascribed to the concept of personalized medicine, which, at least in some iterations, includes routine use of WGS (3). It is essential for the scientific and medical communities to be realistic about the clinical potential of WGS to prevent losing public support for these important endeavors. One need only look to the optimistic predictions made at the time of completion of the first draft of the human genome 10 years ago, claiming that a medical revolution of personalized and DNA-based medicine was imminent. That revolution has largely not yet come to be, and the popular press has begun to promote a view that genetics has failed to live up to its promises (4).

The vast majority of genomic data is, at this time, not medically actionable. Nearly all of the most highly significant genetic associ-

ations resulting from genome-wide association studies for common diseases confer odds ratios too small to be useful in clinical medicine (5). This in no way negates the extraordinary biological insights and additional research that continues to flow from these studies but should caution those who make predictions about the use of such information in clinical settings. Moreover, how multiple risk variants combine in additive, multiplicative, or compensatory ways to inform clinical risk is largely unknown. In addition, data from studies of disease concordance in monozygotic twins suggest that for many common diseases, such as cancer, a negative test result from WGS data would not appreciably reduce an individual's risk relative to the baseline population risk and would therefore not enable meaningful medical intervention (6). The challenge is to identify and validate genotypes via WGS that are robustly associated with disease phenotype and with effect sizes, sensitivity, and specificity that enable counseling about risk beyond what is predicted by traditional clinical factors.

A particular concern is that some of those making claims about the application of WGS may not be considered objective or dispassionate because of their commercial or even academic interests. Despite the public hype about the clinical utility of genomic testing, the small-print legal disclaimers of some of the organizations offering direct-to-consumer genomic testing state that the information is not intended for the "diagnosis, cure, treatment, mitigation, or prevention of any disease or other medical condition or impairment or the status of your health" (7). Genomic testing is claimed to allow one to "personalize your health," and yet the terms of service

Whole-genome sequencing may dramatically alter medicine, but there are obstacles to broad implementation.

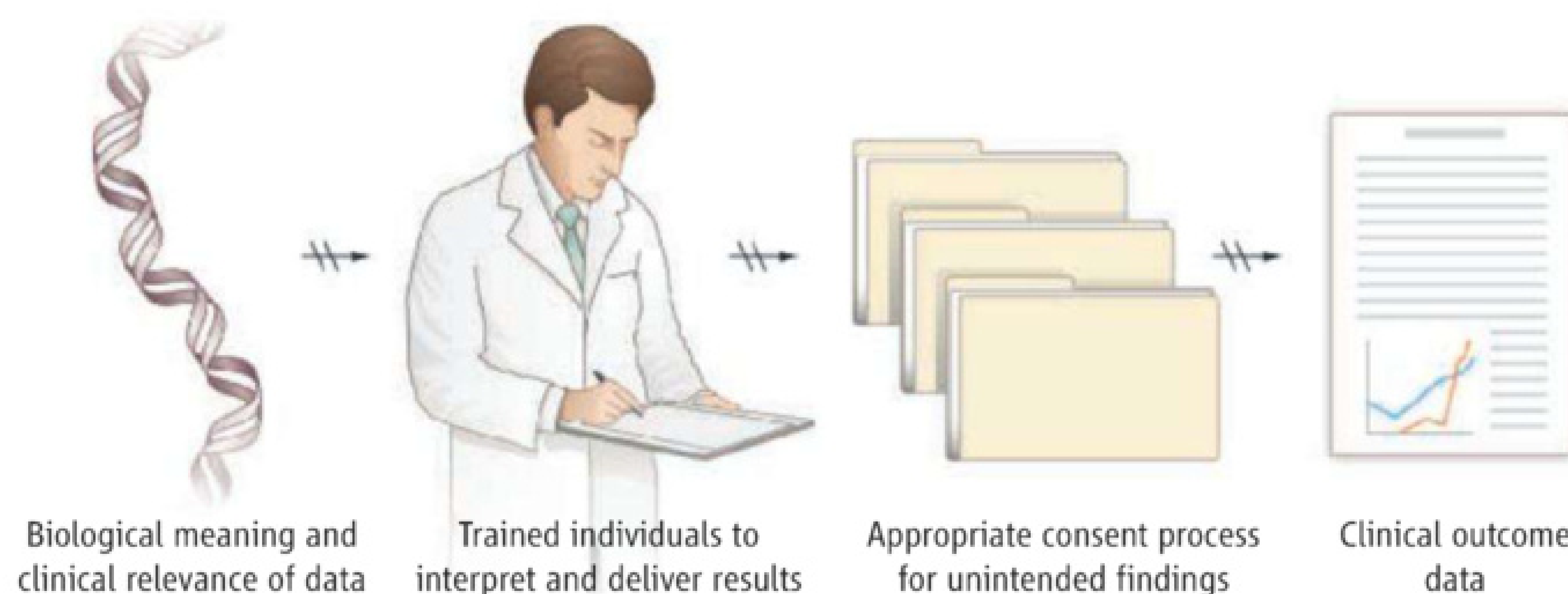
of some tests indicate that the results do not "[recommend] any specific treatment plan, product or course of action" (8). Such contradictory claims only serve to obfuscate the public's understanding of the utility of genomic testing.

In Need of Translation

A major obstacle to large-scale uptake of WGS is the paucity of individuals skilled to interpret and translate genomic sequence data. The bottleneck in bioinformatics analysis and the cost associated with the interpretation of genomic data represent major obstacles. Even the physical storage of these vast data sets poses new challenges (9). Once the sequence data have been analyzed, annotated, and interpreted, we are faced with the profound shortage of translators of these data into meaningful clinical interactions. This includes both genetic counselors and physicians or nurses with sufficient training in genetics. The National Society of Genetic Counselors lists 2307 members in its directory or one genetic counselor for every 135,000 individuals in the United States (10). The small number of individuals who could perform this crucial function will be overwhelmed by the amount of data generated by routine WGS. Each genome is expected to contain ~150,000 novel single-nucleotide variants not currently in the public database dbSNP (11), including 250 to 300 disruptive variants in genes, 50 to 100 variants in human disease genes, and ~20 completely inactivated genes (12, 13). It is estimated that the informed-consent process alone for such testing will take several hours of direct patient contact to review the information relative to likelihood of illness, its implications, and approaches to managing disease for each gene

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Gaps in the delivery of routine WGS of healthy individuals.

under discussion, as well as the potential risks and benefits of knowing versus not knowing specific genomic information (14). We simply do not have sufficient numbers of trained individuals to meaningfully translate these results. The exponential advances in sequencing technology have exposed major obstacles to personalized medicine on any large scale.

Incidental-omics

The chance discovery of variants of potential clinical importance underscores the complex nature of the informed-consent process that will be required for routine WGS. We favor a model in which a patient would specifically consent to learning of categories of variants in specific genes with different clinical implications. One category would be clinically important, medically actionable variants in genes conferring specific risks. Examples would include *BRCA1* and *BRCA2* mutations associated with breast and ovarian cancer, *APC* mutations associated with familial adenomatous polyposis, or pharmacogenomic variants of high predictive value such as the HLA-B*5701 allele (which predisposes to abacavir hypersensitivity). Many patients undergoing WGS may choose to learn of such results because evidence-based interventions are potentially available to reduce risk [i.e., prophylactic mastectomy in the case of *BRCA1* and/or *BRCA2* mutations (15), colonic surveillance in the case of *APC* mutations, or avoidance of abacavir in the case of HLA-B*5701 (16)].

A second, more problematic class of variants would be those that are clinically important but not necessarily associated with a medical intervention, such as mutations in the Huntington disease gene or in *PRNP*, which has been associated with prion disease. Although learning of these variants may have profound personal importance and lead to changes in life-style, such results may have unwanted psychosocial or economic implications. Specific and informed consent is necessary to undergo appropriate counseling for

this class of variants. For patients who choose not to learn of such variants, a “binning” approach could be used in which clinically important but nonactionable variants would be censored from WGS results (17).

Incorporation into Clinical Medicine

Clinical reasoning is fundamentally Bayesian. How the results of a test alter risk is crucially linked to the pretest likelihood that the patient might have the condition of interest. For example, in a patient with clinically diagnosed iron overload, homozygosity for the C282Y mutation in the *HFE* gene is highly predictive of the diagnosis of hereditary hemochromatosis (HH) (18). However, in an unselected population, the C282Y mutation confers only a very low risk of developing clinical disease (19). This speaks to the penetrance of a given genetic variant, as well as the importance of interpreting the variant within a clinical context. Performing WGS routinely in healthy individuals separates the test from the clinical question. This can lead to overinterpretation or false-positives (diagnosing a condition when it is not truly present). An example would be erroneously diagnosing HH based on a mutation in the *HFE* gene in a low-risk individual. Routine WGS could also lead to false-negatives, i.e., missing potentially important findings because absence of a clinical question did not focus the analysis to look deeply enough. Examples of the latter would be failing to identify or correctly interpret functional nongenic variants or complex chromosomal insertions, deletions, or rearrangements.

Last, with few exceptions, there is a lack of data to suggest that genetic testing actually leads to improved health outcomes, a separate issue from the predictive ability of a genetic test. The examples described above of prophylactic mastectomy to reduce the risk of breast cancer in *BRCA1* mutation carriers and avoidance of abacavir in carriers of the HLA-B*5701 allele are notable

exceptions (15, 16). Once a specific genotype has been robustly shown to be predictive of a specific condition, the question of whether knowledge of this risk will enable intervention to improve health remains unanswered. Although early reports of clinical use of WGS have been encouraging (2), new clinical trials and regulatory methodologies may be required to rigorously and efficiently assess whether genomic testing will lead to improved health outcomes. This will be essential before we can legitimately advocate for WGS on a larger scale and will likely be required by payers for reimbursement of such testing.

Minding the Gaps

How will we move forward to overcome the obstacles? WGS has already proved to be an outstanding research tool, and developing publicly accessible databases of phenotype-genotype information will facilitate our growing knowledge of how to apply this information. Guidelines from professional organizations and medical groups will be helpful in guiding appropriate use of this technology. In this regard, the recent guidelines published by the UK National Health Services are a welcome contribution (20). We must train more translators. Clinical training programs must reorganize themselves to train health-care professionals to use genomic testing. We currently face not a technological gap but a delivery gap both to the patient and to our communities (see the figure). Steps to overcome these gaps are urgently needed to fulfill the promises and hopes for the genetic revolution.

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EVOLUTION

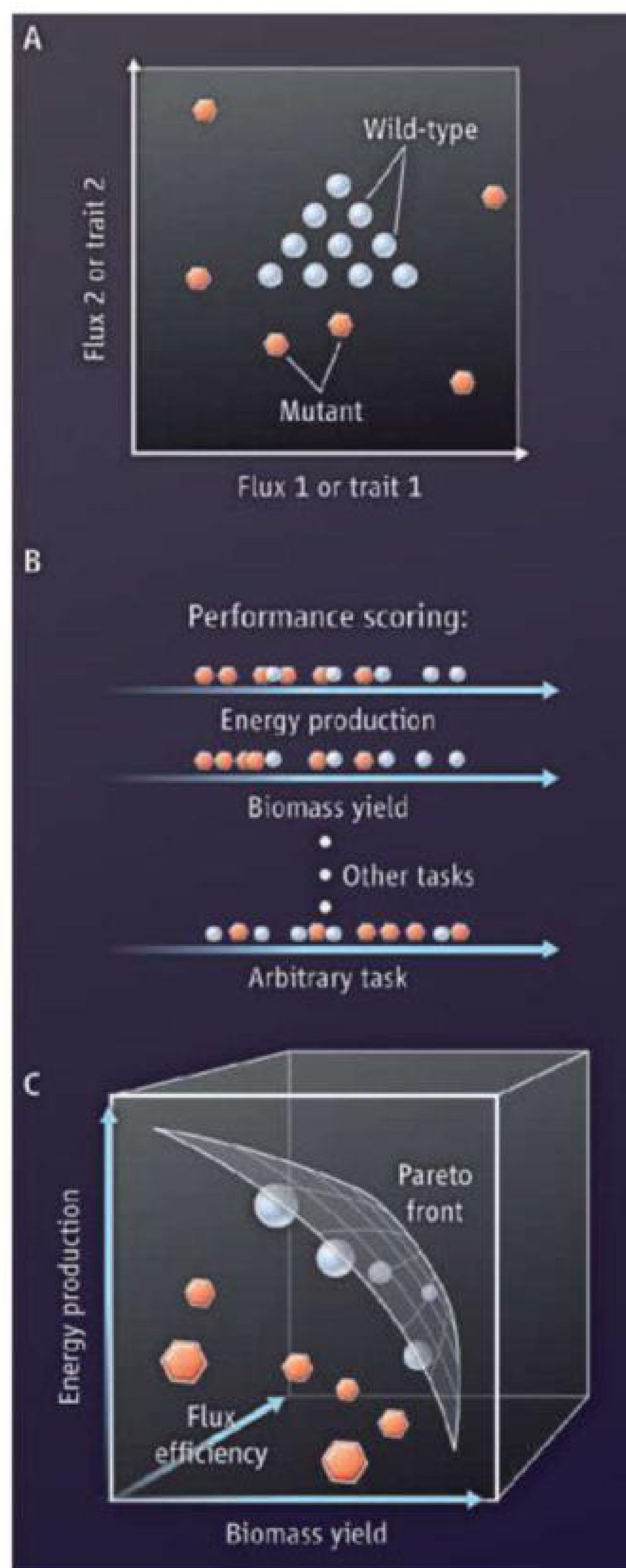
Efficiency in Evolutionary Trade-Offs

Elad Noor and Ron Milo

Cheetahs are the fastest land animals on Earth. But why aren't they even faster? And how did leopards, which live in the same habitat but run only half as fast, survive the competition? Obviously, other conflicting evolutionary factors exist besides speed. Cheetahs are worse tree-climbers than leopards, probably due to their semiretractable claws that are a disadvantage in climbing but an advantage in running. Evolution constantly faces such trade-offs between tasks (or objectives), but it is very difficult to know exactly what these tasks are and to quantify how performance at a particular task affects an organism's overall fitness. Two studies—by Shoval *et al.* (1) on page 1157 in this issue and by Schuetz *et al.* (2)—examine the underlying balance of contrasting objectives in a plethora of biological observations ranging from morphological features like beak and wing shapes to bacterial metabolism. Both studies employ a key concept from economics and engineering—the Pareto front.

Nineteenth-century economist Vilfredo Pareto, best known for his so-called 80-20 rule (80% of the effects come from 20% of the causes), also realized that economies often produce several goods that compete for the same set of resources. A situation is defined as “Pareto efficient” when any further increase in the production of one item would necessarily curtail the production of another. The set of Pareto efficient points is referred to as the “Pareto front” or “Pareto-optimal surface.” By extension, an organism can be said to be Pareto inefficient if its performance at one task (such as running) can be improved without harming any other (such as tree climbing). Because any variant with better or equivalent performance at all tasks would be more fit, it stands to reason that evolved systems tend toward Pareto efficiency. Putting aside historical contingencies and randomness, and assuming that natural selection results in Pareto-efficient species, can empirical data be used to reverse-engineer evolution and infer what the dominant tasks were?

Schuetz *et al.* measured the distribution of fluxes (metabolite turnover rates) for



wild-type and mutated bacteria in many different conditions and scored each flux distribution using a long list of possible tasks [also known as optimization objectives; e.g., biomass production (3)]. They calculated the distance between the wild-type flux distributions and the Pareto front defined by different combinations of tasks. The authors found that a combination of three tasks—maximal adenosine 5'-triphosphate yield, maximal biomass yield, and minimum sum of absolute fluxes—best explains the observed flux distributions for all organisms and conditions (see the figure). Schuetz *et al.* then examined the adjustment in fluxes

A key concept in economics and engineering is used to deduce which traits and which tasks that an organism performs are important for fitness.

At the Pareto front. (A) Morphospace (phenotype space) is depicted in which the two axes are quantitative traits. Each point represents a single organism or species. (B) Scoring performance for a list of objectives (tasks). The position of each organism or species on each of the arrows represents that score with respect to that task. (C) The Pareto front is shown. If the correct combination of tasks is used, the wild-type organism or species will be positioned on the Pareto front, and mutants will be Pareto inefficient. This is an indication that these tasks dominate the organism's fitness.

that occurs when cells undergo a sudden change in their environment. They show that bacteria facilitate the adjustment by choosing a flux distribution that requires only a small modification when switching between environments, although slightly compromising their Pareto optimality in any single environment. The study thus demonstrates how bacterial metabolism can be understood with the concept of Pareto optimality and minimal adjustment.

The method of Schuetz *et al.* uncovers evolutionarily important tasks, but requires scoring performances based on measured organism traits (e.g., fluxes). However, many systems in biology are too complex to currently allow such scoring. Might there be a way to find the dominant tasks by leveraging phenotypic data, which often are more easily obtained? Shoval *et al.* provide a methodology for doing exactly that. Rather than trying to compute the performance directly, they focus on the shape of species' distribution in morphospace (4)—where each axis is a quantitative trait and each point is a species. By assuming only that performance decreases with distance from a small set of specialist phenotypes, called “archetypes,” they demonstrate that species should be arranged in that morphospace within simple geometric shapes—such as straight-line segments or triangles. Data from several studies are consistent with this theory, including measurements of the beaks of Darwin's finches (5). Plotting the different beak shapes of the ground finches in morphospace reveals that they are indeed arranged in a triangle, where one of its archetypes (i.e., vertices) is the beak of the large ground finch, highly suited for crushing large and hard seeds. This theory could help scientists to deduce which tasks

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are important for fitness, by analyzing the behavior of species near the vertices.

Using only basic assumptions, Schuetz *et al.* and Shoval *et al.* provide powerful tools for generating and testing hypotheses about which capabilities are selected for in nature. Future research could apply these methodologies in a variety of biological systems. However, several fascinating questions remain unanswered. Is it true that only

a handful of tasks affect the fitness of organisms, or is this an artifact of our limited experimental data and measurement abilities? Further advancing our understanding of evolution as a multi-objective problem will require deciphering how contrasting goals are combined to define fitness, determining why specific points on the Pareto front are chosen and not others.

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GEOCHEMISTRY

Tracking the Fukushima Radionuclides

Naohiro Yoshida¹ and Jota Kanda²

On 11 March 2011, the Fukushima Daiichi Nuclear Power Plant (FDNPP) lost cooling capability during the magnitude-9.0 Tohoku earthquake and the subsequent tsunami (1). The incident led to severe damage of the plant and the release of large amounts of radionuclides to the environment. Local contamination still prevents over 100,000 residents from returning to their homes. Detailed maps are beginning to provide a picture of the contamination patterns (see the first figure), but as radionuclides migrate and diffuse through the environment (see the second figure), continual monitoring is required to guide remediation and ensure human safety.

Deposition of radionuclides on land mostly stemmed from the release to the atmosphere of relatively volatile fission products, most importantly ¹³⁷Cs and ¹³¹I (2). From 12 March to 6 April 2011, an estimated $\sim 150 \times 10^{15}$ Bq of ¹³¹I and $\sim 13 \times 10^{15}$ Bq of ¹³⁷Cs were released into the atmosphere (3); higher values of up to 50×10^{15} Bq for ¹³⁷Cs have also been suggested, with radioactivity detected throughout the Northern Hemisphere (4).

Airborne monitoring by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) and the U.S. Department of Energy (DOE) from April to November 2011 and concurrent ground-based observations have yielded detailed deposition distribution maps of radionuclides (see the first figure) (5). Due to strong

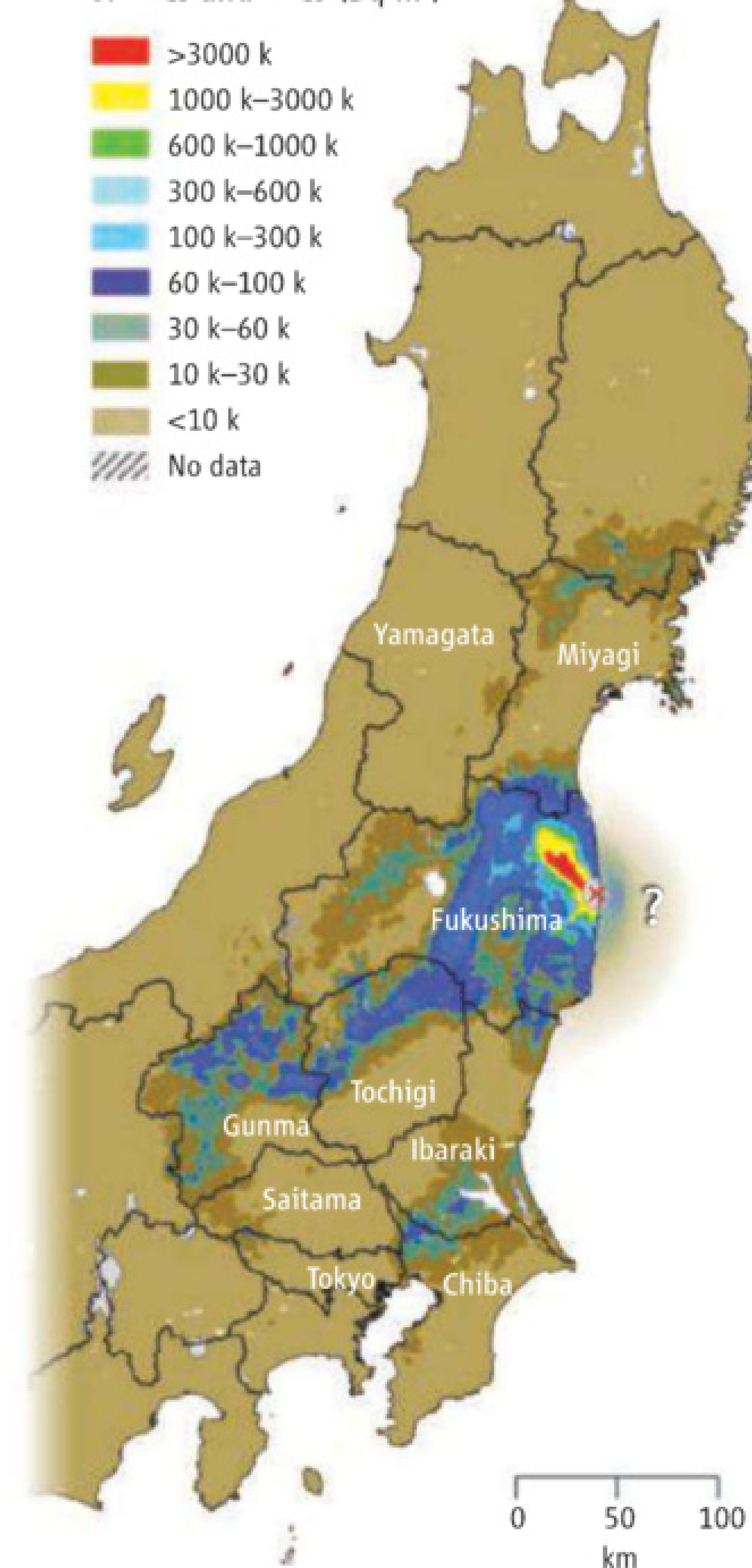
westerly winds prevailing in early spring in this area, about 70 to 80% of the radionuclides emitted from FDNPP were deposited over the western North Pacific Ocean (6). The remainder was deposited on land, especially northwest of the FDNPP (7, 8). The I:Cs emission ratio may have differed between emission events from the damaged reactors; combined with air-mass movements and precipitation, this led to different wet deposition patterns for each radionuclide (9).

¹³¹I has a half-life of ~ 8 days and thus diminishes quickly. However, because of the importance of assessing the radiological dose from ¹³¹I, which could have a large effect on public health of local residents, MEXT is planning a surrogate nuclide ¹²⁹I mapping effort. ¹³⁷Cs has a much longer half-life of ~ 30 years. It adheres strongly to clay minerals and therefore mostly stays in the top 5 cm of soil (10). In nearly half of the 20-km exclusion zone around the FDNPP, ¹³⁷Cs deposition exceeded 600,000 Bq m⁻²; in the most highly contaminated areas, deposition exceeded 3,000,000 Bq m⁻² (5). Rebound and resuspension of aerosols into the atmosphere and of soil particles in water systems can lead to changes in the extent and location of contamination (see the second figure). Such radionuclide transport and redeposition may lead to new radioactivity “hot-spots” on land or in terrestrial waters, and to transport of radionuclides to the sea.

Many other, less volatile radionuclides may also have been emitted to the environment. Plutonium (Pu) deposition has been detected at a site 1.7 km from FDNPP and at several sites between 20 and 30 km from FDNPP (11). Reported ²⁴¹Pu/²³⁹Pu ratios (12)

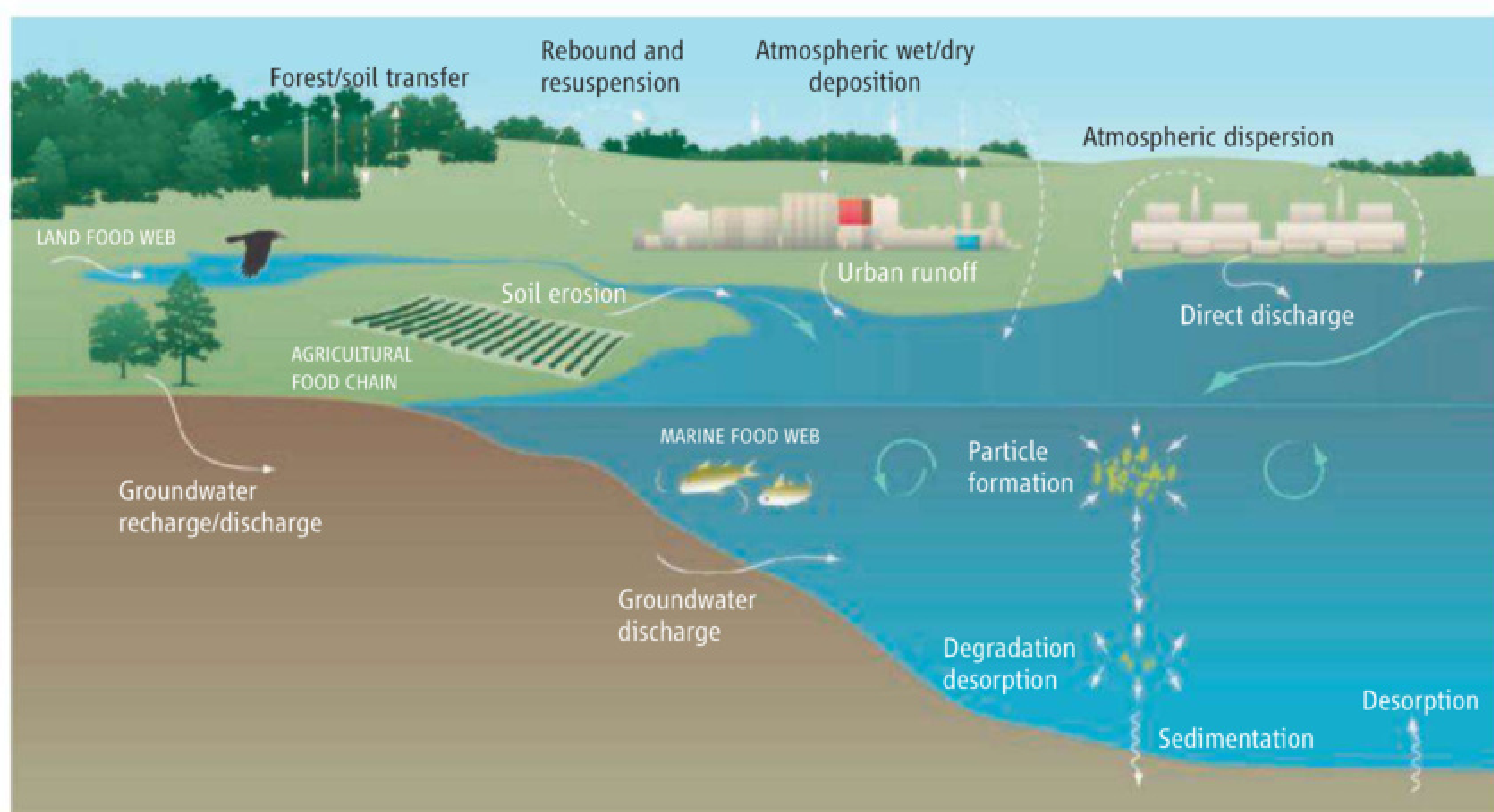
Ongoing radionuclide monitoring and tracking efforts are required following the nuclear accident at the Fukushima Daiichi Nuclear Power Plant.

Total disposition of ¹³⁴Cs and ¹³⁷Cs (Bq m⁻²)



Radionuclide deposition. ¹³⁴Cs and ¹³⁷Cs distribution map [redrawn from (5)]. The distribution of radiocesium in the oceanic system is not readily available, but is estimated to be below 10,000 to 100,000 Bq m⁻² in coastal sediments and waters off Fukushima (5).

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Radionuclide migration. Material cycle processes of radionuclide deposition and migration. Future remediation efforts will require long-term monitoring even at low levels of contamination.

were much higher than those from the global fallout during the era of atmospheric nuclear weapon tests. Long-term dose assessments from Pu and other alpha emitters are important in public health management because of their relatively larger health effect and longer half-lives; such assessments should include ^{241}Pu and its decay product ^{241}Am . The Pu isotopic composition also provides information about the reactor damage. The neutron activity of the damaged core can also be elucidated from a significant rise in the atmospheric level of the neutron-activated radionuclide ^{35}S (13).

Oceanic radionuclide contamination from FDNPP has several sources (see the second figure). When MEXT began to monitor marine radioactivity on 23 March 2011, up to 77 Bq of ^{131}I per liter and 24 Bq of ^{137}Cs per liter were observed in seawater 30 km offshore of the plant. Variable ^{131}I to ^{134}Cs and ^{137}Cs ratios indicated that this material originated from atmospheric deposition (14, 15). Presumed atmospheric deposition also reached remote oceanic stations up to 1900 km away in the North Pacific in late April 2011 (16).

In coastal waters, direct discharge prevailed. Seawater radioactivity peaked at the end of March through the first week of April (14); about 4×10^{15} Bq of ^{137}Cs was released by direct discharge through the end of May (15, 17). From late April, the radioactivity dropped sharply; by June, seawater radioactivity readings were below the detection limit at most sampling points, except in the immediate vicinity of the power plant. This

was, however, partly because of the seawater radioactivity measurement method adopted in the government-organized monitoring; methods with a lower detection limit are readily available but were not used.

Simulation studies (15–17), direct observation (6), and a comprehensive survey by an American vessel in June 2011 (18) show that the contaminated water eventually headed eastward along the Kuroshio Current while spreading and being diluted. The most distinct effect of the FDNPP incident at sea was a high level of contamination within a short period, followed by rapid flushing.

Nevertheless, Cs readings from fish samples taken from Fukushima coastal waters, where commercial fishing is practically banned, still exceed the guideline (19). The radioactivity in fish appears not to follow the rapid decrease in seawater radioactivity. A comprehensive survey of radioactivity in the marine food web is called for.

Although the radiocesium distribution around the FDNPP is relatively well understood (see the first figure), the processes by which it is transferred—e.g., from forest canopy to ground soil and aquifers, terrestrial biota, river and lake systems, and eventually to marine systems—are yet to be depicted quantitatively. For other radionuclides, even the distribution is not well-known. It is thus crucial that monitoring efforts continue, subject to quality control, and that they are complemented by detailed analyses of transfer processes with the aid of high-resolution model simulations. These studies are essential for assessing the external and internal radio-

logical doses, including food consumption, to which local populations have been exposed.

Decontamination in access-restricted areas to which local residents are planned to return is given highest priority in government policy. Decontamination certainly should be pursued until the radiological dose to the public is reduced to a reasonable level, although this level is subject to substantial public debate. The proposed decontamination strategy centers on removal and isolation of topsoil and cleansing of house walls and roofs with water. To evaluate the effectiveness and sus-

tainability of the strategy and avoid recontamination of surrounding regions, these decontamination efforts must be accompanied by tracking migration of radioactivity. Decision-making must be based on scientific knowledge, public disclosure, and comprehensive communication (20).

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GEOLOGY

Understanding Sediments—Reducing Tsunami Risk

Robert Weiss and Joanne Bourgeois

As illustrated on 11 March 2011, tsunamis can cause heavy casualties and hardship and bring about substantial economic loss and environmental change (1, 2). Yet, every tsunami provides new information about the processes that govern inundation characteristics. The primary means for inferring the frequency and magnitude of past, unobserved tsunamis comes from the sediments that they leave behind (3, 4). These deposits can also yield data on tsunami characteristics such as water height, inundation distance, and number of waves—information that is pivotal to forecasting the magnitude of future events.

The study of recent tsunamis has given rise to a proxy tool kit that ranges from classic sedimentary analysis to geochemical signatures of tsunamis (4, 5). Numerical simulations are also a pivotal part of tsunami research. Computer models featuring two spatial dimensions (longitudinal and latitudinal directions) are commonly applied to study offshore and onshore tsunami dynamics. To explore more complex hydrodynamic processes—for example, in the tsunami wave front or during tsunami wave breaking—three-dimensional models are necessary (6, 7), the detail and complexity of which can quickly exhaust computational resources available. Also, for a better understanding of complex currents created by tsunamis in harbors and ports, the incorporation of turbulent effects is pivotal (8). These improvements of model capability are crucial for establishing a more advanced understanding of sediment dynamics during tsunamis.

Advanced numerical simulation techniques have been successfully applied to the 11 March 2011 Tohoku-Oki tsunami to simulate turbulent flow structures near harbors (9) and to the 2009 Samoa tsunami to simulate sediment dynamics in a sediment-starved environment (10). The proxy tool kit has recently been enhanced and examined in the Tohoku-Oki case on the Sendai plain, where the tsunami outran sand deposits (11). However, it remains challenging to apply these techniques successfully and reliably to

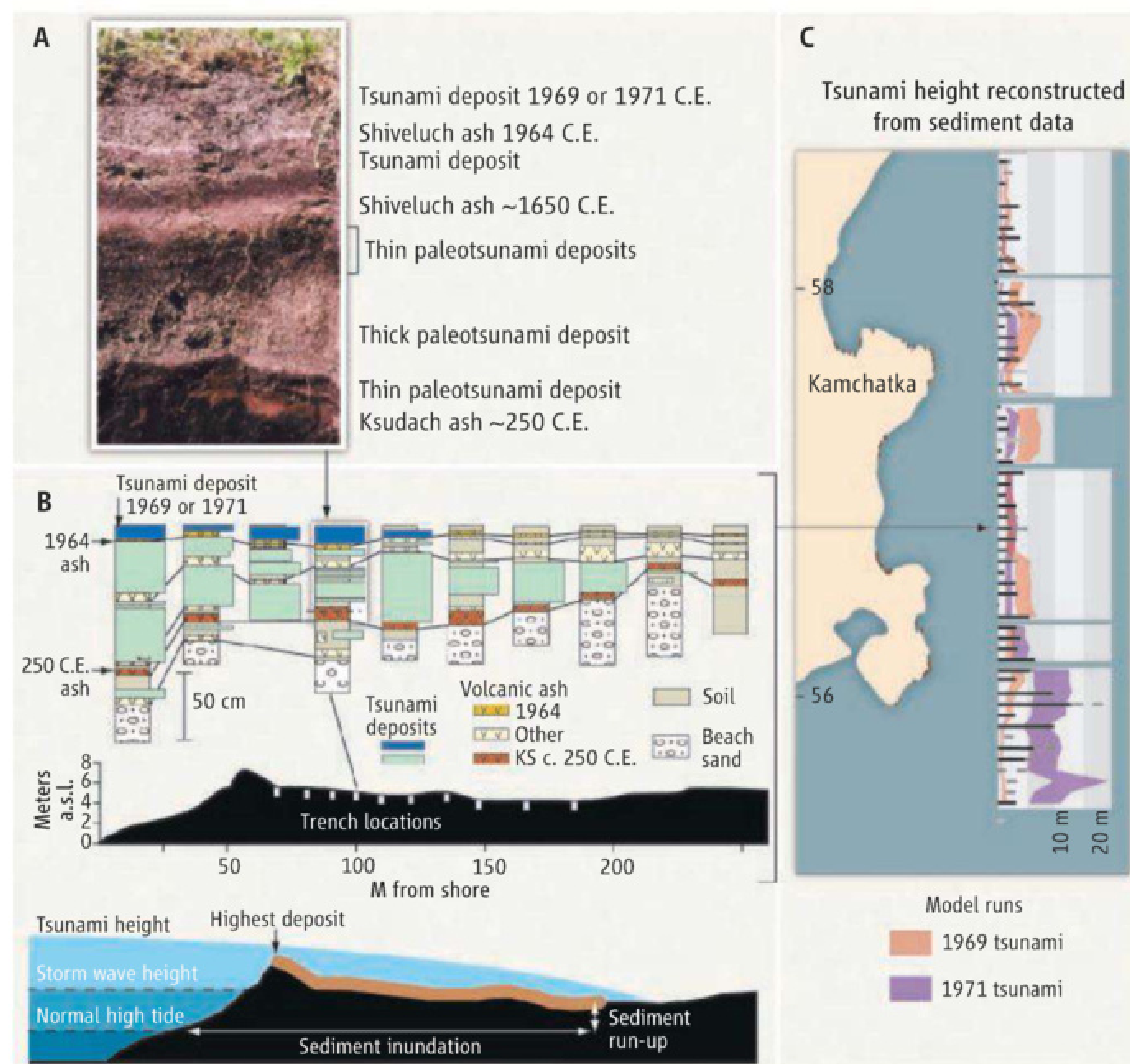
paleotsunami events. The challenge is no better illustrated than by Japan's Tohoku coast, where the C.E. 869 Jōgan tsunami (12) was known both historically and from tsunami deposits, yet the import of this knowledge was still incompletely comprehended (13, 14).

A major avenue toward better understanding tsunami erosion, transport, and deposition is to bridge the gap between field-based and theoretical research. Numerical models (15) have generally used idealized conditions without erodible sediment or sediment-laden flows. Model development would benefit from experiments that focus on the rapidly

Knowledge of the processes that drive tsunami sediment erosion and deposition can help to determine and mitigate tsunami risk.

changing flow and sediment-transport conditions, and the resulting large fluctuations in stress and pore pressure, during tsunamis. To inform theoretical and laboratory research, field surveys after a tsunami should routinely include—as some have already done—observations not only of the spatial distribution and grain-size characteristics of tsunami deposits but also of their setting (such as bed roughness and three-dimensional topography) and sediment sources.

Understanding sedimentary dynamics during modern tsunamis and their manifestation in deposits will help to quantify the



From the field to tsunami modeling. The extent of a tsunami deposit ("sediment runup") marks minimum tsunami runup and can be used to test tsunami source models. This is particularly useful in areas where few historical records exist, such as in Kamchatka, northeast Russia. (A) This excavation profile provides evidence of a tsunami deposit from a tsunami in 1969 or 1971. It is one excavation of 10 from one profile (B) of about 50 along the coast of Kamchatka (C). Tsunami model runs show that only the 1971 tsunami can explain the high runup in the south, whereas the runup in the north is better explained by the 1969 tsunami (C). This is a simplistic case; the future lies in three-dimensional modeling of water and sediment transport. [Figure is adapted from (18)]

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characteristics of causative waves in cases of buried tsunami deposits. Such analysis of repeated prehistoric events can help to project future events and their magnitude. Moreover, the combination of sedimentary and engineering analyses should yield more robust and reliable inferences of potential damage. It is important to estimate maximum flow speed and flow depth for engineering designs that can either withstand tsunami impact forces or undergo controlled failure to dissipate energy. Attempts to retrodict overland flow speed and depth show some evidence of success (16, 17) but are in need of refinement.

Reconstructing past earthquakes from tsunami deposits requires comprehensive mapping and analysis. One tsunami deposit can look very much like another; a sand layer above a time marker (such as an ash layer) may represent two events closely spaced in time or geography. Such events have happened, for example, along the Nankai trough (1944 and 1946); northern Kamchatka (1969 and 1971) (18); Sumatra (2004 and 2005); and the central Kurils (2006 and 2007). Also, the landward limit of a sand or mud layer only represents minimum inundation; moreover, at their landward extent, deposits are thin, subtle, and easily obscured by soil processes.

Coupling of deposit mapping with tsunami source modeling helps to address these problems (see the figure) (18–20).

Another avenue toward understanding tsunami flow processes and their impact lies in collaboration with the storm-science community. Storms and tsunamis have differences, but also commonalities such as onshore flooding and infrastructure damage. Recent events, such as the 2005 Katrina hurricane and the 2011 Tohoku-Oki tsunami, provide an opportunity to study deposits in built environments. Combined sedimentary and engineering analyses improve evaluation of future risk, because quantifiable damage can be linked to observed erosion and deposition.

Real-time forecasts of tsunami events are important, but more effective mitigation is achieved in combination with increased awareness and preparedness in coastal communities. The study of tsunami surface processes and deposits clearly contributes to refinement of risk and hazard assessments. There is also the potential to use the physical record of past tsunamis directly in education and in engineering design of coastal infrastructure. Not only are tsunami deposits a scientific key to past processes but also they are a concrete reminder to coastal residents that “it can happen and has happened here.”

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GEOPHYSICS

A Rogue Earthquake Off Sumatra

Jeffrey J. McGuire¹ and Gregory C. Beroza²

The magnitude (M_w) 8.6 earthquake of 11 April 2012 off the coast of Sumatra is one for the record books. It is far and away the largest strike-slip earthquake in the instrumental record. The M_w 8.2 aftershock that occurred just over 2 hours later is also among the largest such earthquakes. Furthermore, the 11 April mainshock may be the largest intraplate earthquake ever recorded, although the location (see the figure) is consistent with the notion of a wide, diffuse plate boundary that bisects the Indo-Australian Plate near the Ninetyeast Ridge (1). The earthquakes are the latest in a series of large (M_w 8) intraplate strike-slip earthquakes in oceanic lithosphere (2). What do these earthquakes reveal about earthquake

physics, and how might they change earthquake hazard assessment?

The regular occurrence of M_w 8 strike-slip earthquakes, in which adjacent sides of the fault move past one another horizontally, in old oceanic lithosphere presents an interesting problem for fault mechanics. How is it possible to have such large earthquakes on faults that cut vertically through the oceanic plate? In the case of the mainshock, preliminary back-projection results (3) and the aftershock distribution both suggest that rupture may have occurred on multiple faults. Even so, to attain the seismic moment of 9×10^{21} N-m determined by the U.S. Geological Survey (USGS) (4), the product of the average slip and fault area has to be remarkably large for a strike-slip earthquake. The key to understanding this observation may lie in the depth extent of faulting.

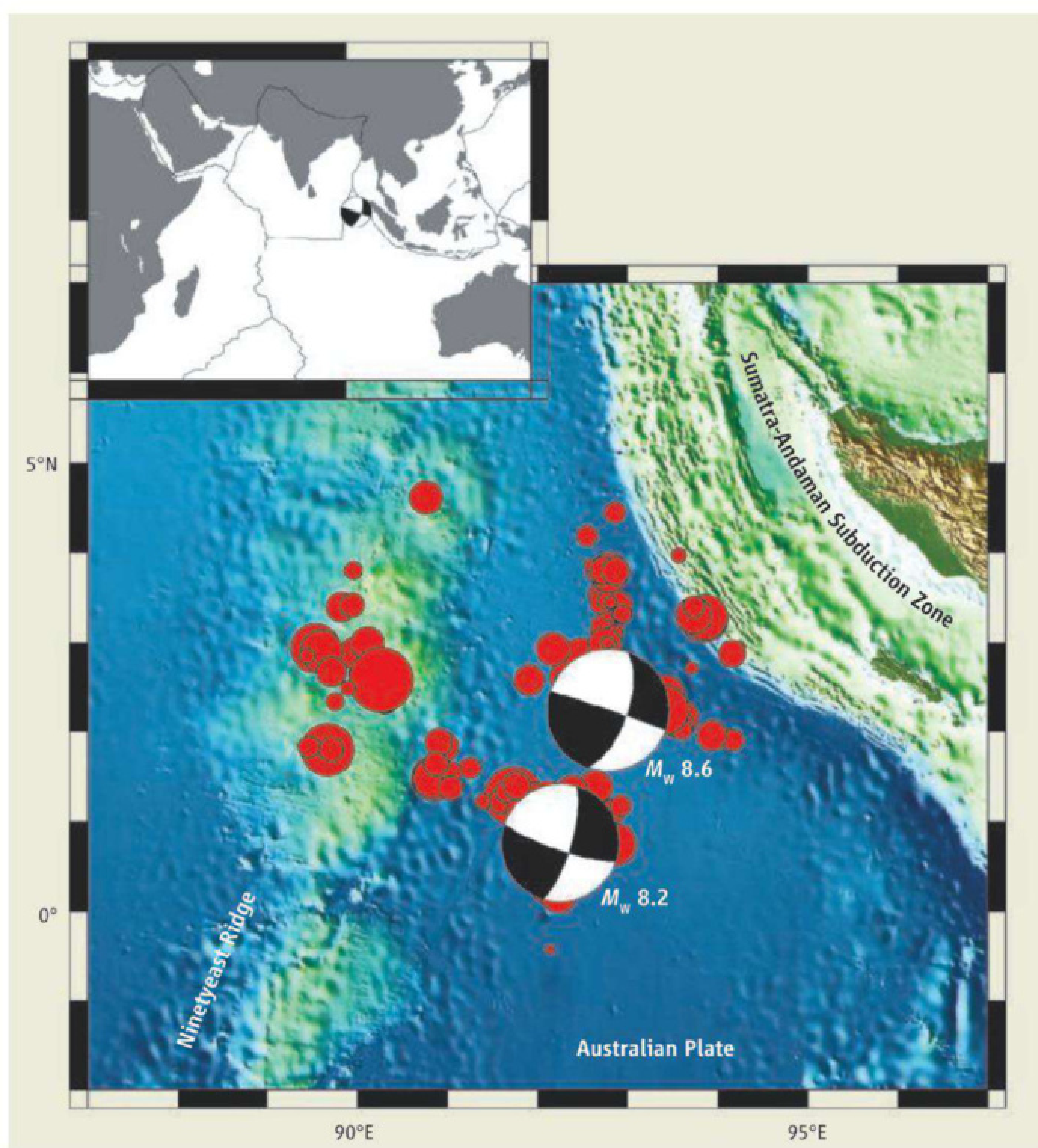
Laboratory studies of olivine (5) and observed earthquake depths (6) had suggested

A magnitude 8.6 strike-slip earthquake within an oceanic plate raises fundamental questions about earthquake physics.

that frictional failure in oceanic earthquakes is limited to temperatures below 600°C. The 11 April mainshock and aftershock had estimated centroid depths (the slip-weighted average of the depth of fault motion) of roughly 40 km and 54 km, respectively (4). Thus, much of the slip in the off-Sumatra earthquakes likely occurred at temperatures of 600° to 800°C, somewhat above where frictional failure is expected. The high pressure and dry conditions at these depths in oceanic lithosphere also make frictional failure unlikely.

Instead, an alternative failure mode may operate that invokes the heat generated by rapid strain in fine-grained shear zones to generate a thermal runaway feedback that results in highly localized zones of viscous failure (7). The thermal runaway mechanism is expected to operate only in the 600° to 800°C temperature range where slip occurred (7). In extreme cases of great pressure (depth) and high slip (large magnitude

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The 11 April 2012 off-Sumatra earthquakes. The mainshock (northern) and aftershock (southern) focal mechanisms are shown at their preliminary Global CMT locations (4). Aftershock locations from the USGS NEIC catalog (15) are shown as red circles. The Sumatra-Andaman subduction zone to the west of Sumatra hosted the M_w 9.1 Sumatra earthquake in 2004 and the M_w 8.6 Nias earthquake in 2005. The Ninetyeast Ridge is the prominent north-south bathymetric feature at the western end of the aftershock zone.

and stress drop), the fault zone may even melt (8). Earthquakes at depths in oceanic lithosphere similar to those of the 11 April events routinely radiate a large amount of energy as seismic waves relative to their magnitude (9). This observation suggests an unusually large drop in the strength of the fault zone during failure relative to frictional events near Earth's surface, further indicating a difference in the physics of rupture that may point to a thermal runaway mechanism.

The focal mechanism of the off-Sumatra earthquake indicates east-west extension. This is remarkable given that the 2004 M_w 9.2 Sumatra earthquake, which occurred just to the east, relieved a tremendous amount of east-west compression. Extensional earthquakes in the aftermath of reverse faulting events are not unprecedented. For example, a substantial normal-faulting sequence

occurred in northern Honshu following the 2011 M_w 9.0 Tohoku-Oki earthquake (10), and outer-rise normal faulting earthquakes have accompanied subduction events in the past (11). However, these latter events are closer to the trench than the off-Sumatra earthquakes, and their tensional mechanisms are thought to be strongly influenced by bending stresses. Given the strength envelope expected to govern stress magnitudes in oceanic lithosphere, it would be remarkable if the 2004 Sumatra earthquake altered the stress field sufficiently to give rise to a strike-slip earthquake of this size and orientation.

The 11 April mainshock caused some damage in Sumatra and was felt as far away as Bangladesh and India; however, there have been no reports of fatalities. As interesting as they may be, large intraplate earthquakes in oceanic lithosphere do not pose

much hazard to life or property. They might, however, inform ongoing debates about the depth of faulting and maximum event size possible for continental strike-slip faulting. Continental strike-slip faults can also host large earthquakes. The largest for which we have instrumental records is thought to be the M_w 8.4 1905 Bolnay, Mongolia earthquake (12). Others, such as the 1906 San Francisco earthquake, have caused widespread devastation. The depth extent of appreciable slip in these earthquakes is thought to be limited to the continental crust. But will that always hold? The 1855 Wairarapa strike-slip earthquake had an incredible 18 m of surface offset (13). Could that be a signature of extensive slip to great depth in something closer to a continental setting?

Another question is how many faults might be involved in a single earthquake. Earthquakes such as the 1992 Landers earthquake have involved multiple, separate faults; others such as the 2002 Denali earthquake have involved multiple kinds of faults. The off-Sumatra mainshock appears to have involved a more extensive network of faults. Is that sort of behavior possible for continental settings as well, or is it peculiar to what may be an incipient plate boundary in the oceanic lithosphere? The earthquake raises these questions but does not answer them. It does, however, compel earthquake scientists to look at past earthquakes and present assumptions with fresh eyes.

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When Less Signaling Is More

Oliver M. Bannard and Jason G. Cyster

A crowded party means lots of sensory inputs and the need to “tune out” distracting signals to have productive interactions. On page 1178 in this issue, Khalil *et al.* (1) suggest that in a crowded environment, B cells too must tune out distracting inputs so that they can be responsive to key interactions.

Antibody affinity maturation occurs inside germinal centers (GCs) within lymphoid tissues and involves B cells testing newly mutated B cell receptors (BCRs) for improved binding to the stimulatory antigen. Only the rare mutant cells that bind better than their sister cells survive, and can either undergo further rounds of mutation and selection, or they can differentiate into antibody-secreting plasma cells or memory cells. But how is antigen binding by the BCR “read out” so that only the high-affinity cells survive? Two nonexclusive models have been considered: High-affinity cells transmit “winning” BCR signals; and high-affinity cells internalize and present more antigen, receiving “winning” T cell help (2, 3).

To test the first model, BCR signaling by GC B cells must be studied. Yet, in contrast to non-GC B cells, investigation of signaling in GC B cells has been limited because of their rarity and propensity to die within hours of isolation. The study of Khalil *et al.* begins

to address this important gap in knowledge.

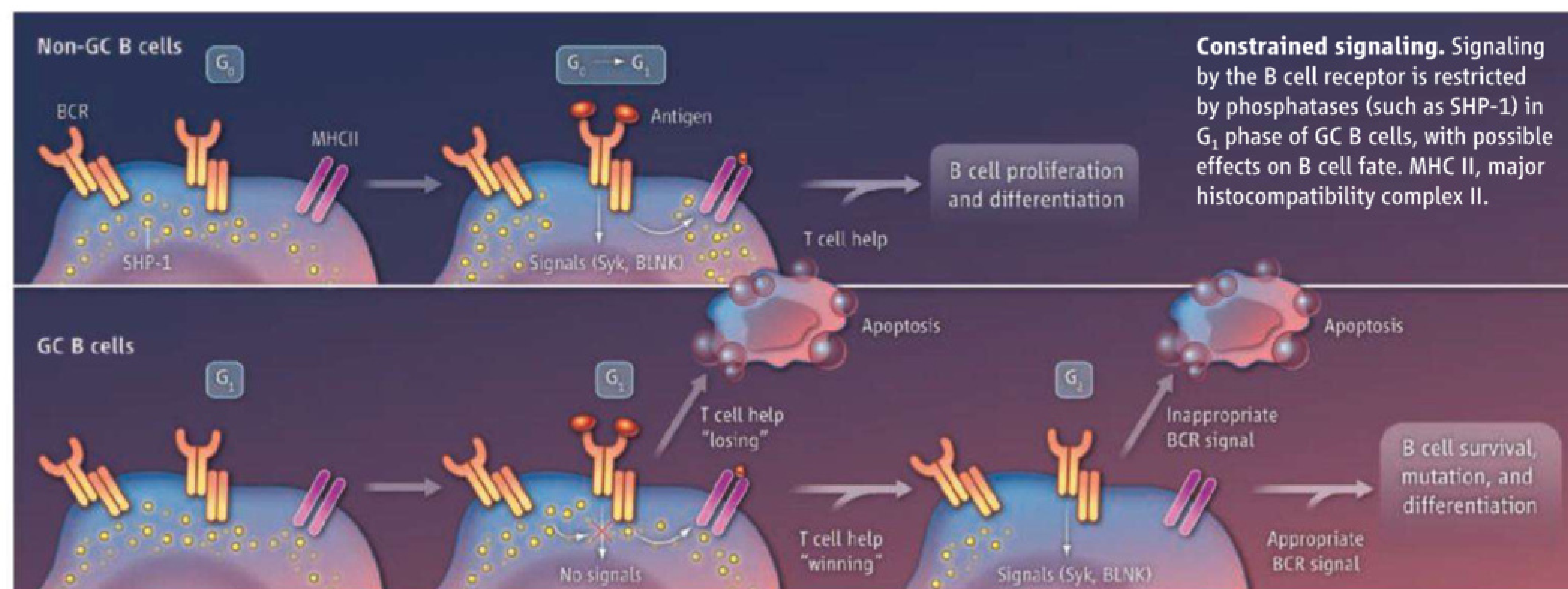
An early step in BCR signaling in non-GC B cells is activation of tyrosine kinases, including Syk, and phosphorylation of adaptor molecules such as BLNK. GC B cells repeatedly encounter cognate antigen, so they might be expected to have elevated “baseline” BCR activity. To examine this possibility, Khalil *et al.* applied an “instant-fix” approach whereby mouse spleen cells were isolated directly into fixative to capture signaling molecules in their *in vivo* phosphorylation state. Unexpectedly, the amounts of phosphorylated Syk, BLNK, and total phosphorylated tyrosine residues were lower in GC compared to non-GC B cells—even after stimulation via surface immunoglobulin M (IgM). The lower amount of surface BCR on GC B cells might contribute to reduced signaling, but comparison of cells with matched amounts of IgM suggested additional constraints on the GC cells. Indeed, when protein tyrosine phosphatase (PTPase) function was globally inhibited, the amounts of phosphorylated Syk and BLNK increased in GC B cells. Surprisingly, in IgM-stimulated GC B cells, the BCR and SHP-1, a major cytosolic PTPase, were codistributed, whereas in non-GC B cells they were segregated (see the figure). Temporal deletion of SHP-1 from B cells led to a fivefold reduction in GC size. Whether this loss was due to exaggerated BCR signaling or reflected a subsequent defect—for example, in antigen presentation—is not yet clear.

Negative regulation of B cell receptor signaling may contribute to B cell selection and cell fate determination in germinal centers.

In a final twist, the authors noted that a minor population of cells expressing high levels of phosphorylated Syk was detectable in the germinal center. They speculated that this might relate to cell division cycle status and indeed found that cells in G₂ phase showed a reduced amount of SHP-1 and reduced colocalization of SHP-1 and the BCR. Thus, phosphorylation-dependent BCR signaling may be reactivated transiently during the G₂ phase of each cell cycle.

In its simplest interpretation, suppression of BCR signaling during the main (G₁) phase of the cell cycle seems to favor the T cell-based selection model, where GC B cells with higher-affinity BCRs endocytose more antigen and receive “winning” amounts of T cell help. But doesn’t receptor internalization depend on signaling? Possibly not, at least not on tyrosine phosphorylation of the BCR and associated molecules that may be most strongly suppressed by SHP-1. Indeed, the BCR complexes internalizing with antigen could be the ones with the least phosphorylation (4), although Syk-dependent signaling may be required for antigen presentation by B cells (5). However, neither of these studies involved GC B cells, which may well differ from non-GC B cells in their antigen-presentation requirements (6).

Although Khalil *et al.* provide evidence for increased negative regulation of BCR signaling in GC B cells, a contribution of BCR signaling to affinity-based selection is not yet ruled out. For example, dur-



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ing encounter with antigen in the germinal center, engagement of the CD19 co-receptor complex may occur. CD19 deficiency is associated with a defect in the GC response (7, 8). Perhaps CD19-mediated amplification of BCR signaling overcomes the phosphatases to influence GC selection events. As for the elevated BCR signaling during G_2 (1), cells in this phase may be enriched in the GC dark zone (3), a region low in foreign antigen and helper T cells (2), which might facilitate negative selection of cells that have acquired affinity for ubiquitous self-antigens (8).

Many GC B cells undergo isotype switching, changing the class of antibody produced. Whether the findings of Khalil *et al.* will hold for isotype-switched B cells will require further studies. IgG and IgE have longer cytoplasmic domains than IgM that include a signaling motif (9). Switching to IgE favors GC B cell differentiation to short-lived plasma cells (10). The relieved BCR signaling during G_2 might also enable the cell to sense what isotype it is expressing and adjust its

cell fate decision-making accordingly.

What might be the basis for the distinct partitioning of SHP-1 in non-GC and GC B cells? The transmembrane protein-binding partners of SHP-1 likely contribute to this process, perhaps redundantly (11). Visualization of SHP-1 in non-GC B cells showed that stimulation via IgM is associated with increased SHP-1 and BCR colocalization (12); whether this difference was due to the use of distinct BCR-crosslinking agents, specific properties of the transgenic B cells studied by Khalil *et al.*, or other factors merits further investigation. SHP-1 also has affinity for membrane lipids and cytoskeletal proteins (13). Clearly, more work is needed to elucidate how SHP-1 is differentially regulated in non-GC and GC B cells.

Why might there be such a big difference in the BCR signaling requirements of non-GC and GC B cells? One possibility is that reduced BCR signaling actually augments antigen presentation. Another is that naïve B cells may depend on strongly amplified BCR signals to

bring them out of quiescence and ready them for cell cycle; GC B cells are already in cycle, perhaps minimizing the amount or type of signaling needed for the BCR to influence cell fate. Although Khalil *et al.* provide important new insight regarding signaling in GC B cells, further questions will need to be addressed if we are to better grasp how antibody affinity maturation takes place.

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ASTRONOMY

Evidence of Things Not Seen

Norman W. Murray

The inventory of known extrasolar planets (planets orbiting stars other than our Sun) has grown explosively in the past 3 years. The explosion relies on detecting the small but distinct decrease in the flux of light from a star caused by the passage of a planet across the stellar disk, an event known as a transit (see the figure). NASA's Kepler telescope, with a photometric precision of ~20 parts per million, has identified more than 2300 extrasolar planetary candidates (1). For comparison, radial velocity surveys, which identify planets by the orbital velocity they impart to their host stars, have found only about a thousand planets since 1995. For the brightest host stars, one can obtain the high-quality spectra that are needed for radial velocity measurements to verify or exclude the planetary nature of the transiting system. Unfortunately, the bulk of the transit host stars are too dim to obtain radial velocities. Enter transit timing variations (TTVs) in the length of a candidate extrasolar planet's year. Since the advent of Kepler, it has

become common to use TTVs to verify the planetary nature of transiting objects in multiplanet systems (2, 3). Recently, TTVs were used to infer the presence of an unseen planetary companion, but with only weak constraints on the orbital period and companion mass (4). On page 1133 of this week's issue, Nesvorný *et al.* (5) take the next step by identifying and characterizing unseen (nontransiting) planets using TTVs.

Transits have long fascinated astronomers. Jeremiah Horrocks predicted and then observed the transit of Venus in 1639. Transits of Venus, as seen from Earth, happen rarely. The next transit will occur on 5 June 2012 and then again in 2117. The same distant observer on a planet orbiting a distant star, in contrast, might see a transit every Venusian year (~225 days), if they lie very close to the plane of Venus' orbit.

Distant observers might or might not see transits of Earth, depending again on how close they are to Earth's orbital plane. However, even if they could not see transits of Earth, they could readily detect the gravitational effects of Earth on Venus (and the Sun). The length of the Venusian year would be seen to vary by 10 min as the Earth alternately

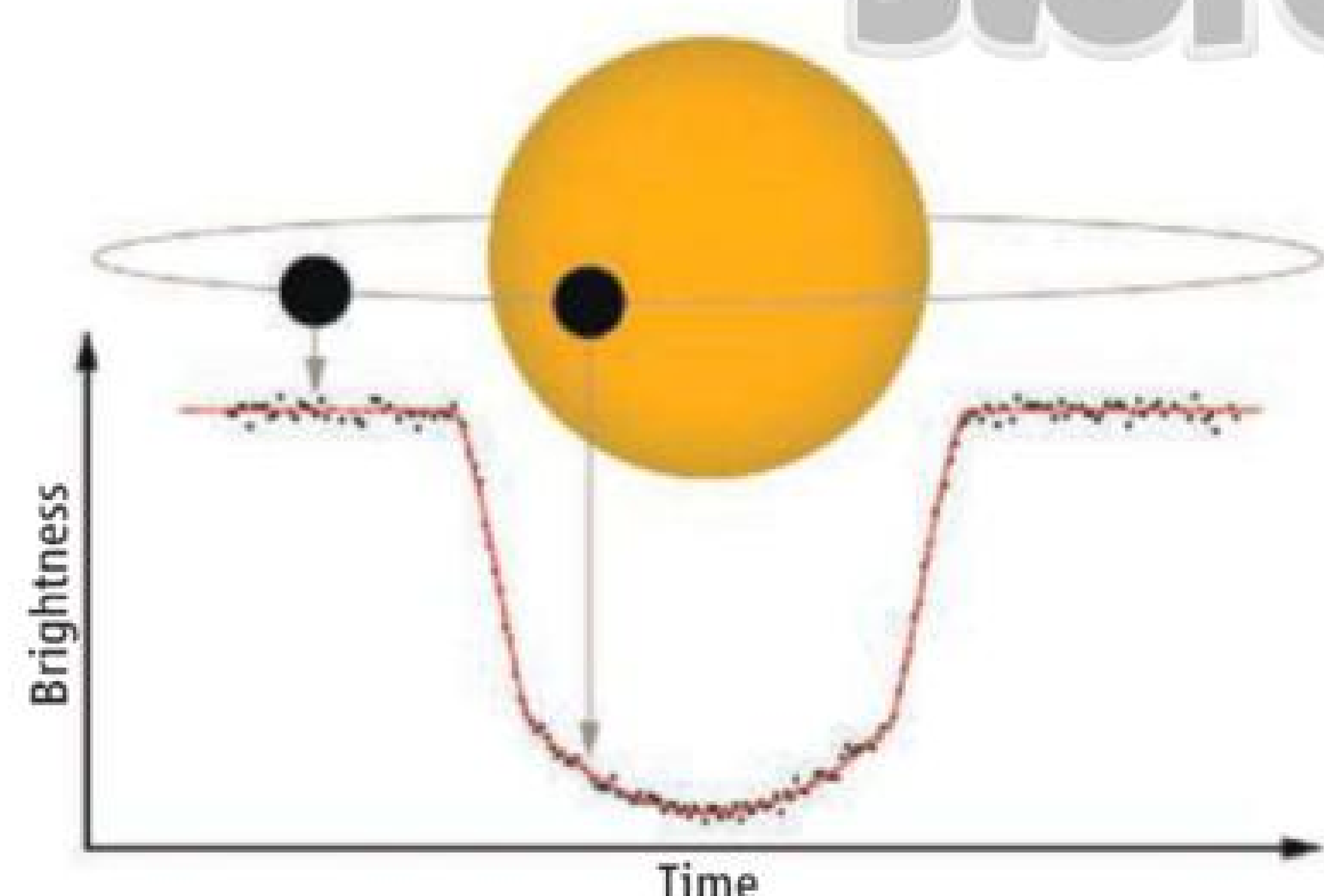
pulls Venus forward in its orbit and then drags it back (6).

The analysis of publicly available data for Kepler-object-of-interest 872 (KOI-872) by Nesvorný *et al.* differs dramatically from that published by the Kepler team. The latter had previously identified a 33.6-day-period transiting planet with a radius similar to that of Jupiter and a mass less than six Jupiter masses (<6 MJ). Nesvorný *et al.* argue that the system harbors two additional planets, a 57-day-period object not seen to transit the star—inferred from the TTVs of the 33.6-day object, with an estimated mass of 0.375 MJ—and a second, previously unreported, transiting object with a period of 6.77 days and a radius about twice that of Earth.

The novelty of Nesvorný *et al.*'s work resides in the identification of the nontransiting body's mass and orbital period. The use of TTVs to find unseen planets, although predicted some 7 years ago (6, 7), has not yielded secure detections before this work.

Are the TTVs due to a second planet or some other cause? Nesvorný *et al.* argue rather convincingly that the former is the case, although their claims will likely face intense scrutiny. Better yet, they make predictions for

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Transit spotting. An illustration of a spatially resolved transit of exoplanet WASP-10b showing both the planet (small black circle, shown at two different times and hence two different points in its orbit) and the star, which is shown as brighter near its center and darker at the limb. As the planet moves onto the disk of the star, it first blocks out the light from the limb, then the more intense light from the center of the star, leading to the characteristic cupped transit shape shown by the red line and data points (9). TTVs, which are not illustrated here [see Fig. 1 in Nesvorný *et al.* (5)], are seen as variations in the time between subsequent transits.

the times of future transits, which will allow for a clear test of their model, because Kepler is scheduled to continue taking data for some time. This is science at its finest, providing solid evidence of things not seen.

The TTVs are obvious in the public data used by Nesvorný *et al.* The length of the transiting planet's 33.6-day year varies by 2 hours, a huge discrepancy. These TTVs are so striking that one wonders why they were not followed up before now by the Kepler team.

Given the large number of promising targets that Kepler has found, the apparent inability to follow up every interesting system should not engender any criticism of the Kepler team; they are simply suffering from their own success. It is clear that many members of the Kepler team have devoted years of their lives to the success of the project, and this work should be rewarded; one way that NASA has done so is by the provision of a proprietary period during which members of the team have exclusive access to the data. However, NASA also specified that the proprietary period have a finite duration. The

case of KOI-872 clearly illustrates the wisdom of NASA's policy.

In terms of learning something about these new planets, we would like to find Earth analogs—rocky bodies with radii of around 6000 km. The best handle on the bulk composition (rocky versus gas) is the density of the planet; measuring the density requires both the radius (from a transit) and the mass (from either radial velocity or TTVs). Of interest, too, is location: How far is the planet from its host star? In particular, if the planet is similar in size and composition to Earth, is the planet orbiting at a distance that might allow for the presence of liquid water? For Earth-like bodies around solar-type stars, liquid water is expected for orbits with periods on the order of 1 Earth year.

Transit observations by Kepler have so far found only a handful of Earth-size bodies orbiting their host stars, all at distances similar to or smaller than the distance between Mercury and the Sun. Based on radial velocity results, it has been estimated that ~25% of solar-type stars possess Earth-size planets with orbits shorter than 50 days (8), so Kepler will find many more such objects. Kepler will undoubtedly announce the discovery of Earth-size planets in the near future,

but before an official announcement can be made, three successive transits are required.

In the longer term, we should be able to probe the atmospheres of Earth-like planets. Transmission spectra of the much larger short-period Jupiter analogs have revealed the presence of heavy elements such as sodium in the atmospheres of several planets. Photometry has been used to constrain the scale height of atmospheres on a number of planets, offering insights as to the atmospheric composition. Similar measurements on smaller planets are currently not feasible but might be within reach of the next generation of extremely large optical telescopes, transforming things unseen into things seen.

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PHYSICS

Speeding Up Quantum Field Theories

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Universal quantum computers in the strict sense do not yet exist, but it is still of fundamental importance to know which problems they can solve more efficiently than classical computers. On page 1130 of this issue, Jordan *et al.* (1) report on such an efficient quantum algorithm that can

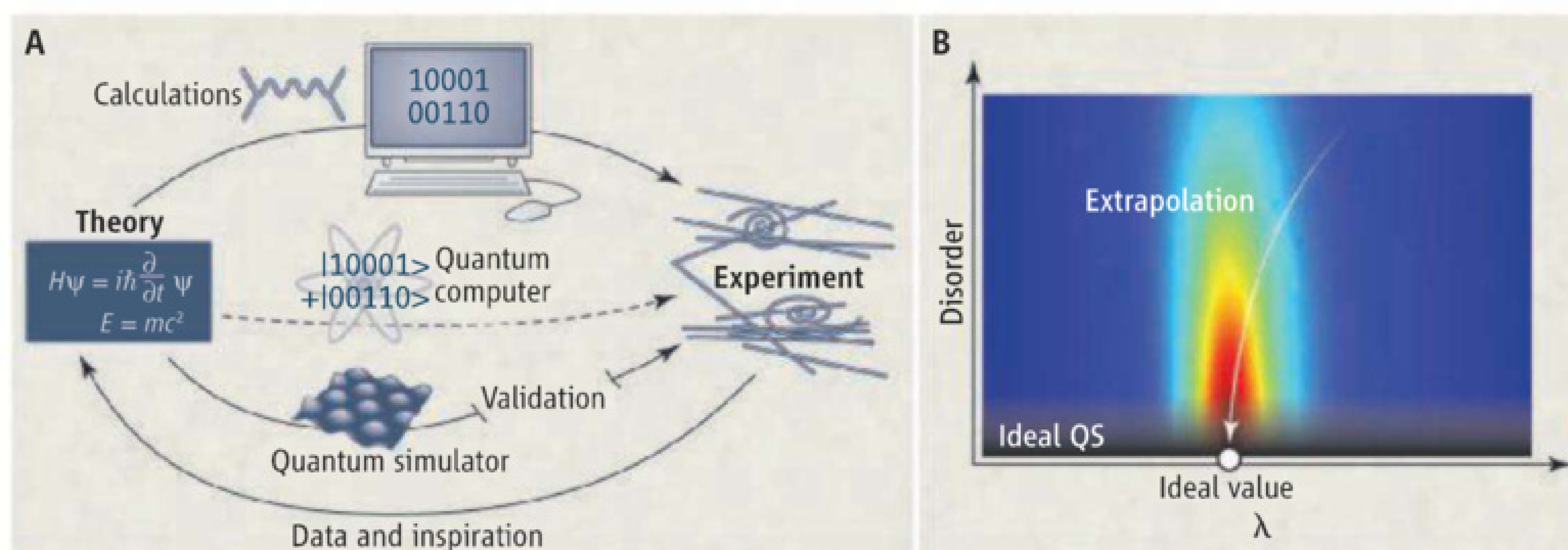
solve the equations of quantum field theory (QFT). These equations reconcile quantum mechanics with special relativity and are crucial for studies of the interactions of fundamental particles.

For classical computers, the complexity class of problems that can be solved efficiently (by which we mean that the running time and resources needed to perform the calculations scale polynomially with the problem size) is termed P (2). Another important complexity class is NP, which includes the set

An efficient quantum algorithm for solving quantum field theory equations can be applied even to difficult cases that include strong particle interactions.

of problems for which, if we know the putative answer, we can efficiently check its correctness. For quantum computers, the class of problems analogous to P, called BQP, are the problems that can be solved efficiently with bounded error. Similarly, quantum analogs of NP problems is the QMA (quantum Merlin-Arthur) class for which the correctness of the answer can be checked (although not computed) efficiently by a quantum computer. Since the seminal discovery of a quantum algorithm for large-numbers factoring (3),

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Quantum simulations of quantum field theories. (A) The methods outlined by Jordan *et al.* would use future quantum computers to greatly accelerate quantum field theory calculations that are now performed classically. It may be possible to implement these methods via analog quantum simulators (Qs) that could be constructed now, but the validation of such devices is still missing. (B) As proposed in (11), one strategy could be to test how the results of an analog QS depend on purposefully introduced imperfections. This example shows the suppression of the correlation length along the phase diagram (spanned by the parameter λ) for a paradigmatic one-dimensional system in which the amount of disorder is varied. An extrapolation of the results at finite imperfections (e.g., to identify the location of a critical point in the phase diagram) can constitute a first step to validate analog QSs and could complement the implementation of the ideas of Jordan *et al.* in such devices.

the community believes that BQP not only includes P but is actually larger.

In 1982, Feynman (4) suggested that quantum computers could be used to calculate the properties of quantum many-body systems. Feynman helped to formulate QFT and invented a specific technique to solve in some regime the equations arising therein—the celebrated Feynman diagrams. However, this technique has a limited range of validity, and in the so-called strong-coupling limit, many of the equations of QFT (notably the ones that deal with out-of-equilibrium dynamics) cannot be solved.

This strong-coupling regime is where a quantum computer could make a huge difference. The constructive quantum algorithm developed by Jordan *et al.* could solve the equations of QFT efficiently and compute predictions that can be compared with the data from particle accelerators. The proposed algorithm is polynomial in the number of particles, their energy, and the desired precision—that is, it belongs to BQP. In the limit of the so-called strong coupling of QFT, the algorithm actually provides an exponential acceleration with respect to the known classical algorithms.

The work is based on three important technical achievements. First, Jordan *et al.* show how continuous fields can be accurately represented by a finite number of qubits whose coordinates form a lattice. This achievement is highly nontrivial, because QFTs are contaminated by infinite values of various quantities that must be cured by using the so-called renormalization and regularization methods, both of which feature naturally in their algorithm. Second, one bottleneck for an efficient implementation of the simulation—the prep-

aration of the initial state—is achieved by a clever preparation of particles in the form of wave packets. Third, the time evolution is split into the action of local quantum gates. This procedure works well for local theories (field theories discretized on a finite spacetime mesh) whose accuracy and convergence must be controlled. Jordan *et al.* prove that they can achieve this control for continuous fields. All of these results are interesting in their own right. At present, the method is restricted to the simplest bosonic QFT. However, we expect it to be generalizable to theories that involve fermions and non-Abelian gauge fields such as the one present in the Standard Model of particle physics that is supposed to describe the real-world phenomenology of strong and electroweak interactions.

Large-scale universal quantum computers, despite massive efforts within various scientific communities, do not yet exist. Thus, the practical importance of the result of Jordan *et al.* might look marginal at this time. However, quantum computers of special purpose, called quantum simulators (QSs), are already being used (5–10). Recently, there has been a true explosion of studies of various experimental systems, such as ultracold atoms and ions, photons in nanostructures, and solid-state systems (such as quantum dots and Josephson junctions) that may serve as quantum simulators of condensed-matter systems or high-energy physics phenomena.

A relevant question is whether the methods of Jordan *et al.* can be reliably implemented on such QSs. Indeed, “Can we trust quantum simulators?” is still an open question (11). Both proposed and realized digital QSs (which so far operate on a small scale with only a few qubits) do not have access to

the full universality and scalability of a universal quantum computer. There, the methods developed by Jordan *et al.* might help to rigorously estimate their limitations and to bound and control their errors.

Analog QSs, in which one attempts to experimentally engineer a system described by the model one wants to address, are currently more common in laboratories. For analog QSs, the question of validation of the results is even more complex, because unlike digital quantum simulators, there exist no known error correction schemes. Still, because of their wide availability, we think that it would be worthwhile to try to adapt the methods of Jordan *et al.*

to this scenario. In this sense, the recent proposal to design various additional tests for the results obtained by analog QSs—for example, by introducing controlled quenched and annealed disorder (see the figure)—could be useful to estimate the bound on errors.

Last but not least, there have been several recent examples where advances in quantum information and quantum computation, such as the understanding of the entanglement structure for relevant quantum states, have been exploited to design more efficient classical algorithms [e.g., the recently developed tensor network algorithms (12)]. These developments give hope that classical simulations of quantum field theories in 1 + 1 dimensions, and perhaps even in some instances of 2 + 1 dimensions, might be efficient (13–15). Again, the techniques developed by Jordan *et al.* may turn out to be useful for such investigations.

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Designing Cell-Compatible Hydrogels for Biomedical Applications

Dror Seliktar

Hydrogels are polymeric materials distinguished by high water content and diverse physical properties. They can be engineered to resemble the extracellular environment of the body's tissues in ways that enable their use in medical implants, biosensors, and drug-delivery devices. Cell-compatible hydrogels are designed by using a strategy of coordinated control over physical properties and bioactivity to influence specific interactions with cellular systems, including spatial and temporal patterns of biochemical and biomechanical cues known to modulate cell behavior. Important new discoveries in stem cell research, cancer biology, and cellular morphogenesis have been realized with model hydrogel systems premised on these designs. Basic and clinical applications for hydrogels in cell therapy, tissue engineering, and biomedical research continue to drive design improvements using performance-based materials engineering paradigms.

Hydrogels, which are three-dimensional (3D) networks composed of cross-linked hydrophilic polymer chains, can be cast into practically any shape, size, or form and can absorb up to thousands of times their dry weight in water (Fig. 1). In medicine, dozens of hydrogel products based on synthetic or natural polymers have had an enormous effect on patient care in recent years. For example, soft contact lenses are typically made from poly(hydroxyethylmethacrylic)acid [poly(HEMA)] (1), and biological adhesives made from reconstituted fibrin or albumin are routinely used in surgical procedures (2). Wound dressings made from alginate polysaccharide have been used for over a quarter century (3), and fillers made from hyaluronic acid (HA) have been used for several clinical indications (4).

New applications for hydrogels have emerged, most notably in stem cell and cancer research, cell therapy, tissue engineering, immunomodulation, and in vitro diagnostics. These applications require more than mechanical and chemical versatility from hydrogels; they require cell compatibility based on biomimicry of the extracellular matrix (ECM) (5). The emerging field of cell-compatible hydrogel materials is therefore defined by design strategies focused on tuning the biological and physical attributes of hydrogels in order to achieve specific interactions and responses from cellular systems. In stem cell research, for example—in which hydrogels are applied to investigate cell fate by regulating key variables of the biomimetic niche—complex patterns, and gradients of chemokines, cytokines, growth factors, and other biophysical properties are systematically designed into the bioartificial stem-cell microenvironments to mediate cell activity at the molecular level (6–8). For hydrogels that facilitate controlled drug release or optimized pharmacokinetics, the porous structure of the material

and its affinity to its therapeutic payload can be chemically controlled without attenuating inherent physicochemical compatibility to natural tissues (9). In regenerative medicine, in which hydrogels are used to hierarchically organize cells into tissue-like structures, the architectural and/or molecular cues can be engineered with spatial and temporal presentations that mediate multicellular morphogenesis (10).

Over the past 15 years, the challenges of designing complex hydrogel systems have been

aided by major breakthroughs in synthetic polymer chemistry (11), 3D molecular patterning techniques (12), and biomimetic rational design approaches that are founded on the basic principles of cell and molecular biology (10). These advances have set the framework for overcoming some of the enduring challenges in applying biomedical hydrogels to additional areas of science and medicine. This review covers the design principles being applied to engineer cell-compatible biomedical hydrogels, focusing on biophysical and biochemical manipulations of 3D hydrophilic networks for controlling interfaces at the cellular and subcellular scales (Fig. 2). A number of recent publications review hydrogels in terms of their application and composition (6, 9, 13–17). Here, we highlight the important role of performance-based engineering concepts in advancing the development of customized hydrogels that contain a set of desired properties based on specific molecular building blocks.

Designing Hydrogel Molecular Building Blocks

A cell-compatible hydrogel is characterized by its ability to control specific molecular interactions at the cell-material interface. These include biological interactions such as receptor-ligand complexes that mediate cell adhesion and mesenchymal migration, bound or soluble molecule interactions facilitating proteolytic biodegradation or transcriptional events that govern cell phenotype, as well

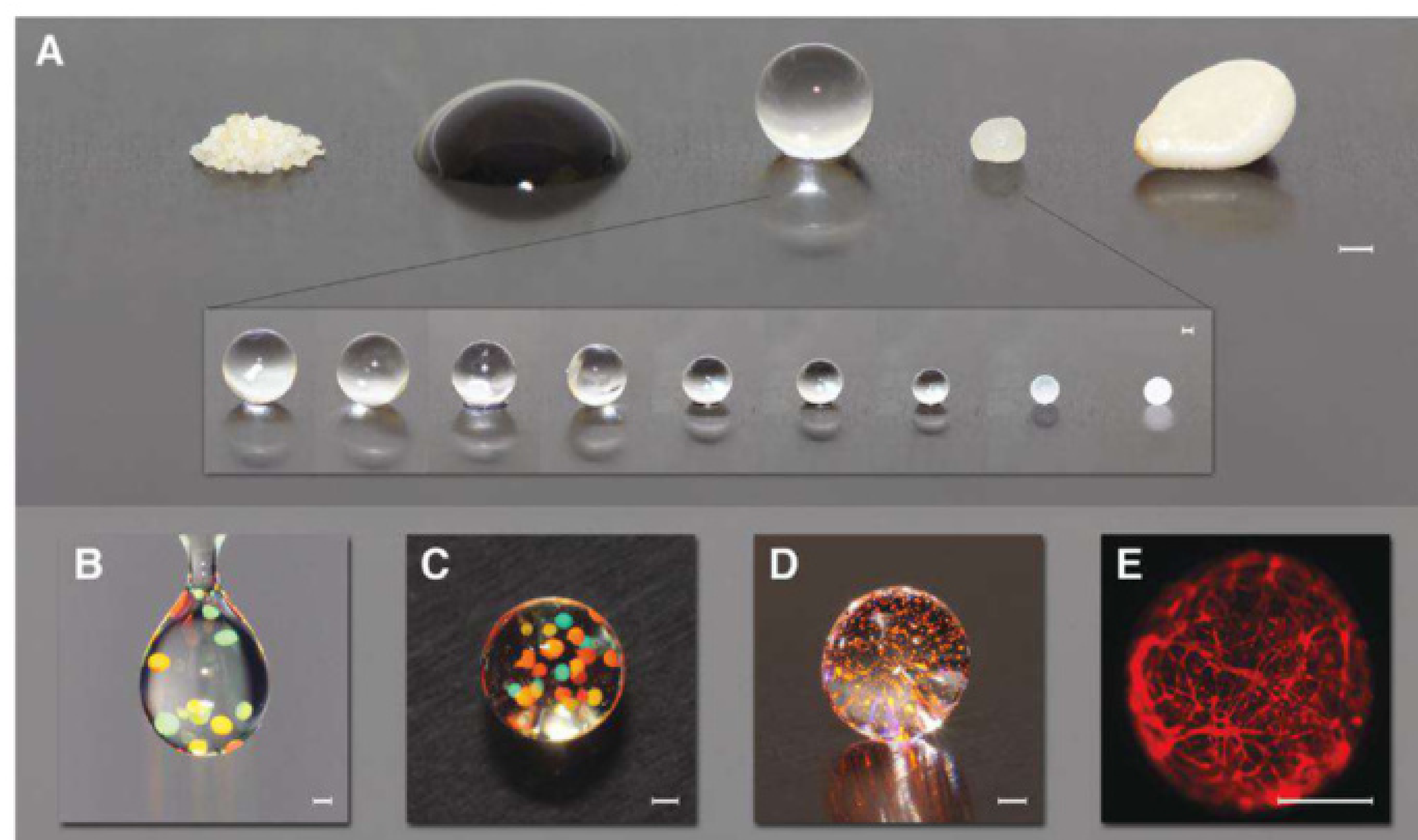


Fig. 1. Hydrogels can be cast into practically any shape, size, and form. (A) Polymer powder (far left) added to a drop of water (left) and cross-linked results in the formation of a hydrogel (middle). Even if the hydrogel network is dehydrated (right), it still retains its overall shape. A sesame seed (far right) is shown for scale. (Insert) The stages of the dehydration process are shown from left to right. (B) Water-borne microgels in suspension containing several different immobilized molecules (color-coded) are shown in a single drop of water so as to highlight the possibilities for injectable hydrogel drug delivery. (C) A transparent microgel containing smaller color-coded microgels is shown to highlight new “gel-in-gel” experimental tools for biomedical scientists. (D) A microgel encapsulating fluorescently labeled cells is shown to highlight potential uses in cell delivery for tissue regeneration. (E) Encapsulated fibroblast cells thrive in a semi-synthetic PEG-fibrinogen microgel, illustrating how highly compatible milieus used for studying cell behavior may one day replace conventional cell culture paradigms in cancer research, stem cell, and development biology. Scale bars, 500 μm .

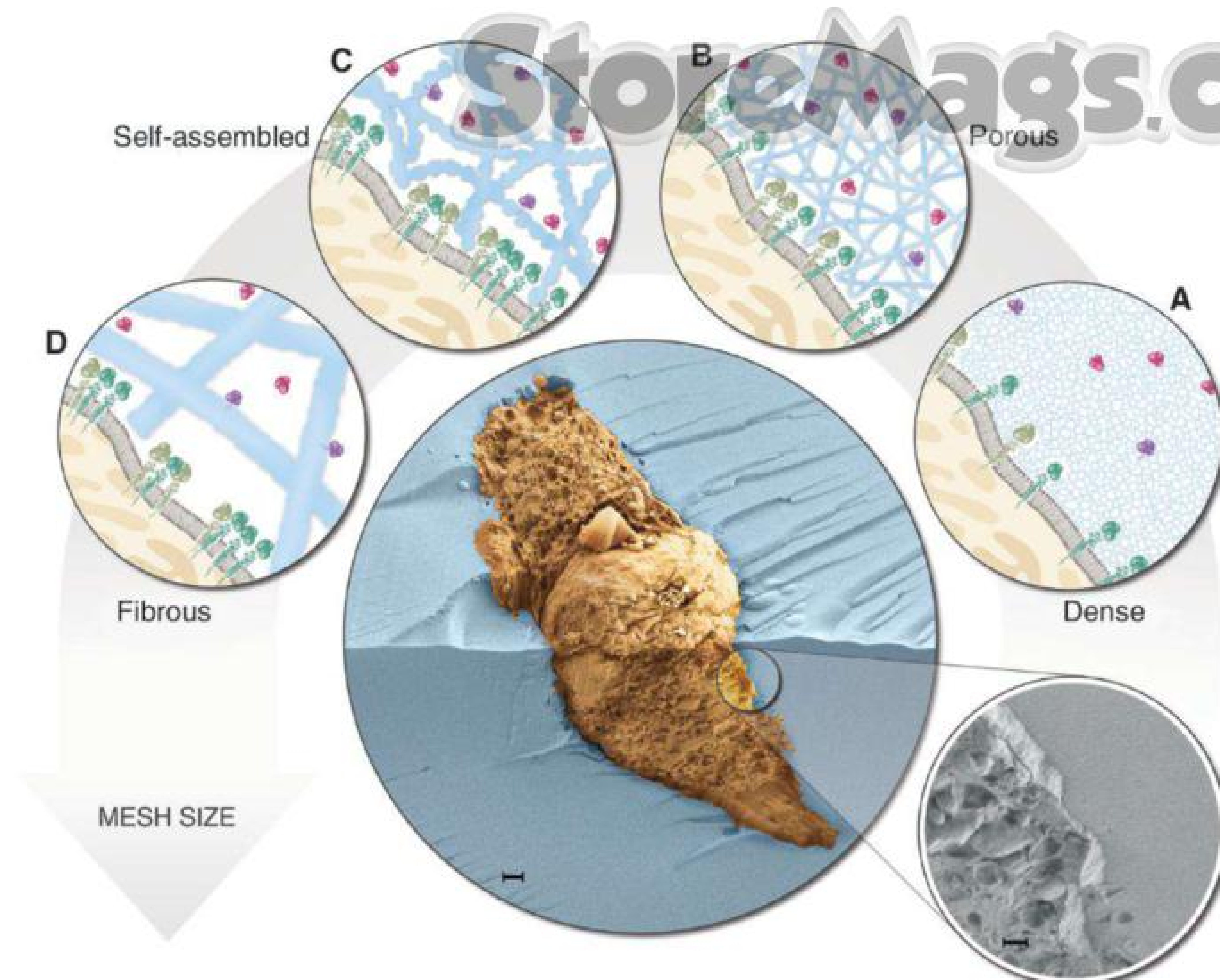


Fig. 2. Interface between cell and hydrogel. A false-colored high-resolution cryogenic scanning electron micrograph depicts the 3D interface between a fully hydrated hydrogel (blue) and an encapsulated cell (brown). Scale bar, 1 μm . The insert (bottom right) shows a magnified view of the cell-hydrogel interface, highlighting the cell membrane in the hydrated gel phase. Scale bar, 300 nm. (A to D) The four other inserts illustrate the cell membrane-hydrogel interface (blue, polymer network; white, void space) in different types of hydrogel structures. Cell receptor proteins (green) lie on the cell membrane (gray), whereas soluble bioactive molecules (red) and tethered bioactive factors (purple) lie within the hydrogel mesh. The length scale of the swollen polymer network can be used to control the transport of factors as well as receptor-ligand interactions and even cell motility. For example, hydrogel structure may facilitate amoeboid cell motility within micrometer-sized pores (D), but not through nanometer pores (A). Similarly, self-assembled and fibrous structures [(C) and (D)] of bioactive hydrogels enable focal adhesion contacts between cells and the network through receptor-ligand clustering, whereas in the dense or amorphous structures [(A) and (B)], these interactions are far less favored because of the spatial distribution and confinement of receptors and ligands (10).

as focal adhesion interactions with compliant or rigid substrates to transmit mechanical stresses to cells. Designing the molecular structure of a hydrogel to control these spatial and temporal events enables their use for guiding cell response in vivo and in vitro. Many features that control cell response, which have been isolated from part of a cell's in vivo microenvironment, can be built into a hydrogel using a top-down rational design.

Two seminal studies in the late 1990s demonstrated the top-down approach using recombinant protein domains that are responsible for the mechanism in which natural proteins undergo reversible temperature-induced gelation. Tirrell and co-workers designed hydrogels that undergo reversible physical cross-linking based on shifts in temperature or pH, using genetically engineered protein domains containing coiled-coil regions (18). Kopecek and co-workers developed similar stimuli-responsive hydrogels based on grafts of engineered coiled-coil or beta-sheet protein domains with a synthetic polymer backbone (19). They showed that the engineered hybrid system exhibited biomimetic self-assembly, as well as control over

physical properties afforded by the synthetic constituent.

Many cell-compatible hydrogel materials have since been modeled on these seminal concepts, most notably the proteolytically responsive synthetic hydrogels pioneered by Hubbell and co-workers (20). They used poly(ethylene glycol) (PEG) macromeres that are cross-linked by oligopeptides that mimic collagenase substrates found in natural ECM proteins. The end-linked PEG-co-peptide hydrogels were formed by Click chemistry, in which a Michael-type addition reaction between the di-thiolated oligopeptides and vinyl sulfone groups on the PEG is carried out in the presence of cells and/or tissues. The chemical cross-linking reactions produced strong elastic gels in mild conditions and ensured high in situ cell survival. This methodology also represented a breakthrough in that it demonstrated the ability to transform an otherwise inert synthetic hydrophilic polymer network into a cell-responsive biomaterial that could undergo controlled protease-mediated dissolution. Other studies have combined a variety of synthetic polymers and protease

substrates—showing that almost any synthetic hydrophilic polymer milieu can be designed to facilitate controlled cellular degradation and invasion. Other biomimetic features isolated from bioactive domains in natural proteins have been used in similar fashion, including cell-adhesive integrin-binding domains (21), controlled release affinity binding domains (22), and transglutaminase cross-linking domains (23).

Some applications may require more extensive biomimetic features from the backbone constituents of the biomedical hydrogel. Certain stem cells, for example, require extensive biochemical stimuli inherent to their natural ECM niche for proper differentiation (6, 7, 16). These stimuli are difficult to replicate with synthetic biology, and a semi-synthetic approach may appropriately accommodate the discrepancy between the completely natural versus completely synthetic hydrogel microenvironments. The semi-synthetics use proteins or polysaccharides conjugated to low-molecular-weight hydrophilic polymers as the main building blocks of the hydrogel (13). The protein/polysaccharide provides biomimetic features, and the synthetic polymer provides important control features for regulating mechanical properties, molecular structure, and other physical attributes of the material.

One such family of semi-synthetic hydrogels is based on the covalent conjugation of linear or branched, nonionic hydrophilic polymers with reconstituted ECM proteins such as fibrinogen, collagen, or albumin (24). The liquid precursors to these hydrogels are synthesized by means of Michael-type addition under reducing conditions with acrylate-functionalized polymers such as PEG, whereas the formation of an elastic gel is accomplished by a light-activated chemical cross-linking. The biological consequence of conjugating the ECM proteins does not alter the inherent compatibility of these native components, and the physical properties of the conjugated polymers are retained or enhanced in the protein-polymer adducts. Shachaf *et al.* demonstrated this with reverse thermal gelation (RTG), a liquid-to-solid phase transition associated with increased temperature of certain amphiphilic polymers in solution. By conjugating fibrinogen to a triblock copolymer composed of a central hydrophobic chain of poly(propylene oxide) flanked by two hydrophilic chains of poly(ethylene oxide) [Pluronic F127 (BASF Corporation, Florham Park, NJ)], the RTG properties of the fibrinogen-Pluronic adducts were enhanced severalfold over the Pluronic alone while still retaining fibrin-like bioactive properties for 3D cell culture (25).

The semi-synthetic biomaterials are now routinely synthesized for biomedical research and clinical applications, including conjugates of HA, alginate, and chitosan (3, 15, 26). Conjugation in these materials has been achieved through stepwise copolymerization of the hydrophilic polymers with protein or polysaccharides, Schiff-bass formation reactions, disulfide bonding, free-radical-initiated copolymerization by using peroxides or

a fenton reagent, photo-initiated free-radical copolymerization, or metal-free Click chemistry such as Michael addition (11). Although less defined than the synthetic ECM analogs, the semi-synthetic ECM hydrogels are relatively easy to manufacture, can be reproduced in large quantities and provide a more reliable material as compared with natural ECM hydrogels.

Although a top-down approach is beneficial, it has its limitations; for instance, it is difficult to precisely control spatial bioactivity given the structural complexity of natural protein domains. An alternative bottom-up approach provides the opportunity to control hydrogel molecular structure by arranging elementary chemical motifs together to give rise to a system possessing controlled yet complex patterns or gradients of bioactive factors, as well as other biophysical properties. The basis of this methodology is functional chemical features whose structure-function relationships are well characterized and which are easily implemented into the hydrogel's cross-linking

methodology. Some well-established chemical reaction schemes have therefore been recently adapted for the mild conditions often required with in situ formation of biomedical hydrogels (27).

Chemical reactions used for the immobilization of reactive macromolecules or localizing cross-linking reactions can be performed by using tightly regulated light-activated initiation either with traditional photolithographic techniques or more sophisticated approaches, such as multiphoton laser scanning lithography (12). Photolithography has been widely used for creating patterns in various hydrogels and, more recently, for patterning cell-laden PEG or alginate hydrogels with multiple functionalities by using free-radical photopolymerization (28). Burdick and co-workers developed a multiple-mode cross-linking methodology in which an ultraviolet (UV) light-induced radical cross-linking reaction morphologically confines 3D cell cultures in spatially controlled nondegradable stiff regions of an otherwise degradable HA-peptide hydrogel (29). The

stiffer UV cross-linked regions in the hydrogel alter differentiation patterns of mesenchymal stem cells (MSCs) and thus may enable the development of niche microenvironments where heterogeneous cocultures are propagated through predefined stiffness and biodegradability.

Patterning techniques with submicrometer-scale resolutions have also been adapted for cell-laden hydrogels based on the concepts reported by Kawata and co-workers using multiphotonic photopolymerization (30). This methodology is applied to a pre-cross-linked hydrogel containing functionalized photo-labile moieties designed to accommodate either immobilization or dissociation reactions initiated by femtosecond laser pulses in the near-infrared range (31). Anseth and coworkers used this approach to creating predefined microchannels within nondegradable or degradable synthetic PEG gels using photolabile cross-linker molecules made with nitrobenzyl ether cross-linking moieties (32, 33). The nitrobenzyl ether cross-links dissociate upon exposure to light from a multiphoton laser scanning microscope system, reducing the local cross-linking density of the hydrogel and giving way to physical tracks for cell invasion via contact guidance.

Hydrogel Structure and Properties

The interrelationship between the hydrogel processing, structure, properties and performance underlie the fundamental design rationale for most biomedical applications. With cell-compatible hydrogels, this interrelationship is complicated by a performance criteria characterized by a multitude of molecular interactions at the cell/material interface (Fig. 3). Therefore, the design should focus on those salient hydrogel features that give rise to the desired properties most suitable for the biomedical application, including transport properties (such as sustained release), tissue interactions (such as bioactivity), and chemical stability (such as degradability). In principle, most features can be engineered into a hydrogel; however, here we will focus on five important properties for biomedical applications: degradation, bioadhesion, bioactivity, transport (for example, controlled release of bioactive molecules), and mechanical properties.

Biodegradation of hydrogels is essential for biomedical applications that require controlled resorption in vivo and/or local dissolution to facilitate cell morphogenesis and motility. Hydrogels have the ability to undergo local or bulk dissolution based on a number of mechanisms (such as hydrolysis, proteolysis, disentanglement, or environmental triggers); engineering the spatiotemporal aspects of this presents a challenge. Bulk hydrolytic resorption with specific temporal events in the body, such as bone regeneration, can be achieved by controlling the amount of hydrolytically labile cross-links in the polymer network, resulting in better tissue repair. Patterson *et al.* created such resorbable bioactive implants for bone repair using photopolymerized HA modified with different amounts of glycidyl methacrylate

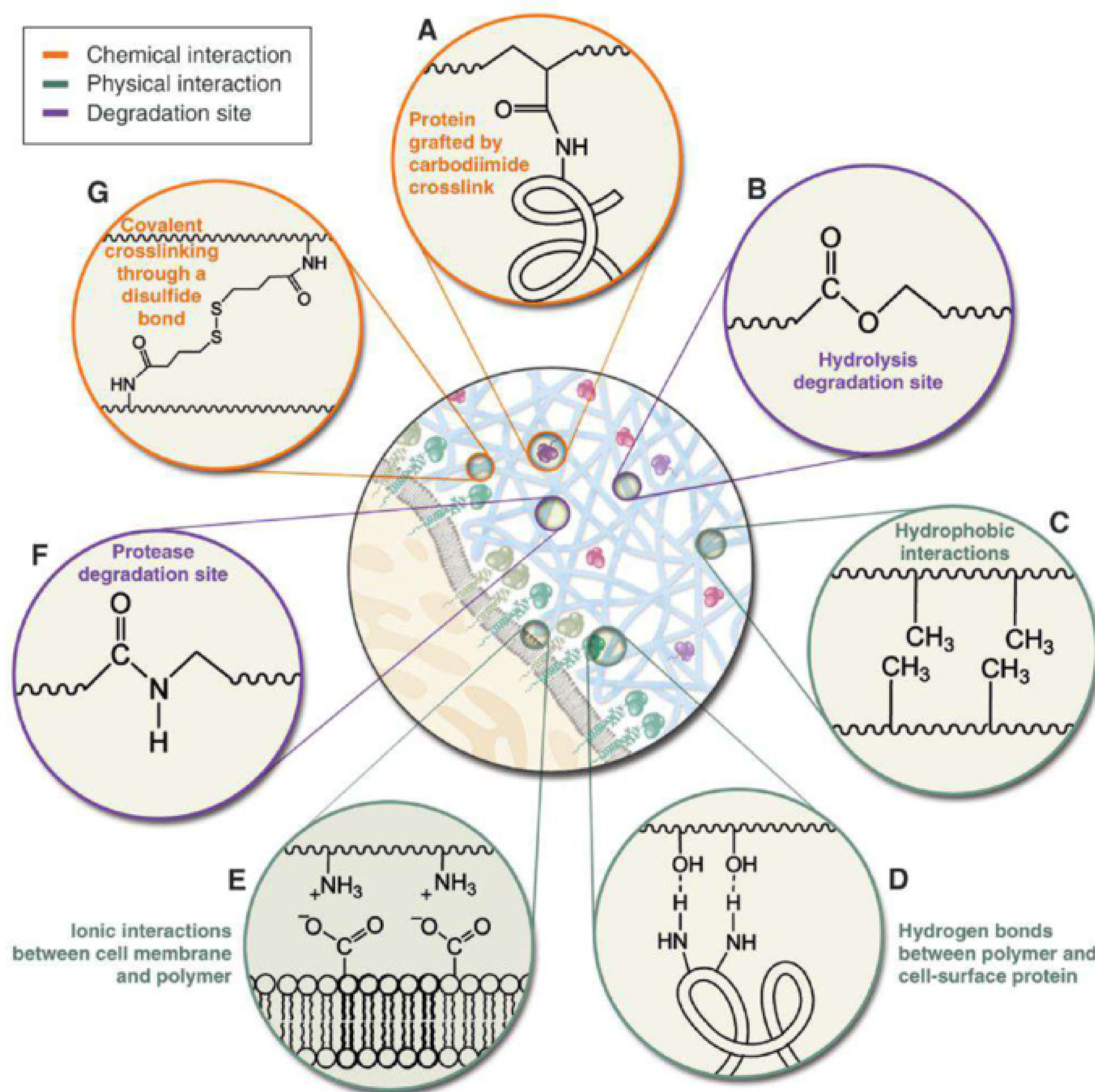


Fig. 3. (A to G) A multitude of chemical interactions underlie the complexity of a cell-compatible hydrogel and its cellular interface. When a hydrogel's polymer chains are held together by irreversible covalent bonds (chemical interactions), the network is relatively strong and stable. When the dominant connections between the polymer chains are reversible molecular entanglements or physical bonds (physical interactions), the network is generally less robust. Degradation can be designed into the hydrogel by introducing either proteolytic or hydrolytic moieties into the backbone of the polymer network. Bioactivity can be designed into the hydrogel by immobilizing growth factors and/or small molecules onto the backbone polymer, or by designing the polymer to facilitate physical interactions with cellular and extracellular biomolecules.

(GMA) cross-linkers. The relative concentration of GMA on the HA was proportional to the hydrogel resorption rate in vivo, and this in turn affected the organization of the new bone formation (34).

Cell-mediated hydrogel degradation can provide a more physiological control mechanism for both the removal of the provisional matrix and the liberation of matrix-bound bioactive factors (10). Strategies using cell-mediated control over degradation use a peptide-polymer hydrogel design with cross-linking oligomers that are known substrates for collagenases, gelatinases, and other matrix metalloproteinases (20). A large number of oligomer sequences that are known to be responsive to these cell-secreted proteases have now been characterized and can be synthesized and incorporated into the material design. In tissue repair of a critical-size rat calvarial bone defect, bioactive hydrogel implants designed with oligopeptides engineered to facilitate an in vivo resorption rate that coincides with the normal repair timeline outperformed all other material variants (35).

Bioadhesion, an important property that allows cells and tissues to adhere to hydrogels has enabled their use as tissue adhesives in surgical repair or as inductive scaffolds for tissue regeneration. Although some hydrogels such as fibrin or collagen inherently exhibit bioadhesive properties, most other natural and synthetic hydrogels do not. Bioadhesive features can be engineered into a hydrogel network by using linker molecules that enable covalent or non-covalent molecular interactions between the implant and its surroundings, including cell-adhesive oligopeptides derived from fibronectin's central cell-binding domains (FNIII 9-10) (21). Cell-adhesion modifications to hydrogel scaffolds have been used effectively to promote enhanced osteogenic differentiation of MSCs for bone repair (21), to provide an essential foothold for neurite outgrowth in axonal regeneration (36), and to understand the regulatory role of mechanotransduction in stem cell fate determination (8).

Tissue-adhesive modifications to hydrogels can further improve performance of cell-delivery scaffolds by stabilizing the in vivo location of the graft. For example, Messersmith and co-workers developed a catechol-based modification to a PEG hydrogel implant in order to improve extrahepatic islet transplantation for managing type I diabetes mellitus (37). The hydrogel modification, using a catechol moiety [3,4-dihydroxy-L-phenylalanine (DOPA)], is derived from the tethering chemistry that allows mussels to adhere to wet organic surfaces (38). Under oxidizing conditions, DOPA rapidly forms cross-links when bound, for example, to an end-functionalized star-PEG precursor. In addition to participating in the nontoxic cross-linking reaction of the PEG hydrogel, the catechol moiety forms strong covalent interactions with nucleophiles such as thiols and imidazoles found in organic substrates (ECM). The effective immobilization of the islet-containing catechol PEG hydrogels on the surface of the

liver of diabetic rats enabled revascularization with minimal inflammation and permitted effective glucose management comparable with the gold-standard intrahepatic islet delivery.

Bioactivity in hydrogels is instrumental for materials that are called on to mediate specific biological events in the body based on endogenous cell recruitment, local morphogenesis, and controlled cell differentiation. Many of these events can be induced by using exogenous growth factors that are delivered with spatiotemporal control (7). However, hydrogels do not inherently sequester growth factors and thus fall short of precisely controlling the sustained or localized bioavailability of their payload. In vivo, growth factor bioavailability is tightly regulated by nonspecific associations between the factor and ECM proteoglycans [glycosaminoglycans (GAGs)] through affinity binding domains (10). Using strategies premised on such non-covalent interactions with polymeric building blocks, design modifications to hydrogels have recently been used to improve the localized growth factor availability (39). For example, Martino *et al.* modified fibrin hydrogels with an oligopeptide derived from a non-GAG growth factor binding domain that was isolated from the 12th to 14th type III repeats of fibronectin; the peptide-modified fibrin hydrogels required far less growth factor than unmodified hydrogels to achieve regeneration in either chronic wounds or critical-sized bone defects (22). These concepts have evolved to include short heparin-binding peptide domains that can sequester cell-secreted proteoglycans, such as endogenous heparin. Murphy *et al.* immobilized such peptides onto materials in order to take advantage of endogenous heparin binding and consequent growth factor immobilization (40). Another prevalent approach to sequester bioactive factors within a hydrogel network involves covalent growth factor immobilization. However, affinity-based linker peptides may be preferred because they circumvent loss of biological activity typically associated with chemical cross-linking of proteins.

Transport of hydrophobic/hydrophilic molecules is an important property of a hydrogel that can benefit therapeutic techniques requiring sustained drug release or triggered pharmacokinetics. For example, tumor chemotherapy may be far more effective if drug molecules were targeted to and sustained in the tumor site, or if a drug is released only after it has been internalized within the cancer cells. Depending on the properties of the therapeutic drug, a hydrogel's porosity (mesh size) may be used to regulate the drug's availability by controlling its diffusion through the polymer network (Fig. 2). The mesh size of a typical cell-compatible dense hydrogel network in the swollen state is no less than 5 nm and far greater than the characteristic size of most small drug molecules (41). For relatively large protein or peptide drug molecules, the hydrogel structure and mesh size (~5 to 20 nm) can be engineered to limit mobility and modulate release kinetics (Fig. 2A). In order to achieve sustained release

of small drug molecules by using size exclusion, hydrogels must use immobilization schemes that entrap these molecules in dense physical structures within the network (mesh size < 1 nm), including in self-assembled nanostructures, layer-by-layer constructs, liquid crystalline nanostructures, and polyelectrolyte complexes (41). Premised on these concepts, Grinstaff and co-workers designed expansile hydrogel nanoparticles for targeting in vivo tumor suppression in a subcutaneous mouse cancer model with the small anticancer drug paclitaxel (42). The hydrophobic polymer-drug nanostructures, which are stable in water at physiological pH levels, are designed to release the drug only after a pH-triggered transition into a hydrophilic hydrogel network occurs inside the mildly acidic endosomes of the tumor cells. This triggered release of paclitaxel was effective in preventing tumor growth in vivo using far lower doses than are required with systemic administration of the drug.

The mechanical properties of a hydrogel can convey important physical cues to cells through mechanotransduction pathways that mediate tissue homeostasis, morphogenesis, cell growth, contractility, differentiation, and pathophysiology. As an example, Weaver and co-workers controlled the modulus of collagen hydrogels to confirm that ECM rigidity can affect the malignant phenotype of mammary epithelial cells through focal adhesion assembly mediated by either ERK activation or Rho activity (43). With new insights into the mechanical basis of tissue regulation emerging, a number of design strategies have evolved to improve control over a hydrogel's mechanical properties. Young and Engler used a slow in situ chemical cross-linking reaction of HA-PEG hydrogels to accomplish time-dependent hydrogel stiffening that mimics temporal events associated with cardiomyogenesis. They engineered the timing of the Michael-addition reaction between thiolated high-molecular-weight HA and PEG-diacrylate cross-linker to recapitulate embryonic stiffening of cardiac muscle in order to show that the temporal pattern of stiffening enhances in vitro cardiac myocyte differentiation (44). Gong and co-workers developed a method for overcoming the inherent limitations of a hydrogel's mechanical properties (such as low strength) using physical interpenetrating double-networks (DNs) (45). The DN hydrogels exhibited extremely high toughness when, for example, they combined a rigid polyelectrolyte gel, poly(2-acrylamido-2-methylpropanesulfonic acid), with a flexible neutral poly(acrylamide) gel. Applying this concept to a cell-compatible but inherently weak polymer such as reconstituted collagen, they produced DN collagen-poly(*N,N'*-dimethyl acrylamide) gels with an order of magnitude increase in fracture stiffness while still retaining more than 90% water content.

Performance-Based Application of Biomedical Hydrogels

Many of the early cell-compatible hydrogel systems lacked essential features, mainly those that can concurrently control material properties,

biodegradation, and bioactivity. Material engineering design principles have overcome some of these limitations, and with these advances, new applications for cell-compatible hydrogels uncovered seminal scientific discoveries, as exemplified by the pioneering work of Discher and co-workers (46). Here, Engler *et al.* used polyacrylamide hydrogels modified with a thin collagen layer and mechanically tuned through radical polymerization cross-linking to document an important responsiveness of MSCs to their substrate elasticity (47). This research represented a major advance not only because it showed the profound influence of ECM mechanics on stem cell differentiation, but also because it ignited a pursuit toward identifying other material properties that can potentially control cell fate.

Discoveries based on hydrogel technologies have also affected translational research in regenerative medicine. Take, for example, the evidence presented by Blau and co-workers that documents how adult muscle stem cells on compliant hydrogel substrates can be propagated in vitro with higher efficiency, leading to better in vivo engraftment (48). These stem cells would normally lose their pluripotency and undergo massive cell death within the first weeks of culture on rigid plastic culture dishes (elastic modulus > 106 kPa); however, when cultured on chemically cross-linked bioactive PEG hydrogels tuned to 12 kPa elastic modulus (and containing immobilized laminin and cell-adhesive oligopeptides), the cells showed signs of self-renewal and were engrafted with substantially better integration in a muscle implant model.

The encapsulation of living cells in a hydrogel milieu is still one of the principal challenges of adapting these materials for further advancing tissue regeneration or studying cell biology using 3D cell culture techniques (49). Cell encapsulation has not lived up to its full potential using existing hydrogel technologies partly because each type of cell requires its own specific encapsulating microenvironment with cell-specific material properties and spatially controlled bioactive features. In order to begin to address these limitations, Anseth and co-workers combined photo-patterning and cyto-compatible Click chemistries so as to improve spatial control of microenvironments in synthetic polymer hydrogels that support protease-mediated 3D cell invasion (33). Here, two orthogonal Click chemistries were used: a Huisgen cycloaddition reaction to cross-link a multi-arm PEG-tetra-azide with di-functionalized oligopeptides (protease-sensitive oligomers), and a secondary photo-activated thiolene coupling chemistry for site-specific biomolecule conjugation (for example, cell-binding oligopeptides). For proof-of-concept, they were able to show that only those niches engineered with a combination of protease sensitivity and cell-adhesive functionality enabled 3T3 fibroblasts to achieve spindled 3D cell morphologies.

Elucidating the specific bioactive requirements of highly specialized cell types (such as cardiac, bone, or liver) and designing hydrogels with

complementary spatial features is still an arduous process. Furthermore, many advanced hydrogel designs will require more than just one or two of the aforementioned properties to mediate complex biological events such as cellular morphogenesis, differentiation, or self-renewal. Branching epithelial morphogenesis, for example—which is in part regulated by complex in situ geometries and soluble factors (such as transforming growth factor beta-1)—should be investigated with bioactive and spatially patterned 3D hydrogel constructs. To do this, Bissell and co-workers used micropatterned collagen hydrogels engineered with cellularized cavities in the form of curved tubules, bifurcated tubules, and fractal trees to show that geometry has an instructive effect on morphogenetic development (50). The important insight from such studies underscores the vast potential of using cell-compatible, custom-designed hydrogel systems in basic and applied scientific research.

Hydrogel Outlook

Hydrogels are already having a dramatic impact in many biomedical fields. For instance, doctors can treat injuries with experimental hydrogels, and stem cell researchers can make discoveries using hydrogels in place of tissue culture plastic (Fig. 1D) (51). As hydrogels improve through better design, their influence is likely to expand to other areas of science and medicine. For example, complicated drug regimens may be replaced with simple injectable microgels (Fig. 1B), systemic chemotherapy may be replaced with hydrogel-based cancer drug treatments (42), and viral gene therapy may be replaced with gene-delivery hydrogels. Beyond biology and medicine, hydrogels may affect the fields of biotechnology, pharmacology, and biosensors by providing solutions for large-scale protein production, drug-screening techniques, and individualized chemosensitivity assays. As an example, drug developers can test antitumor drugs in vitro by encapsulating tumor cell spheroids in protease-sensitive, bioactive hydrogels that more closely mimic physiological tumor growth conditions. Another unfulfilled opportunity for hydrogels is in biotechnology, in which cell manufacturing and protein production in large scales can be aided by encapsulating microgels that enable suspension cultivation of most anchorage-dependent cell types. Whether the objective is protein production or cell expansion, the use of encapsulating microgels would enable the transition from less efficient roller bottle cultures to efficient industrial suspension bioreactors. Thus, as the field moves to use new hydrogel designs, it gains the ability to develop sophisticated hydrogels for these and practically any other application.

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Structure of a 16-nm Cage Designed by Using Protein Oligomers

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Considerable effort in bionanotechnology is aimed at designing protein molecules that will self-assemble into higher-order structures. Design targets include finite structures, such as three-dimensional cages, and materials that extend by growth in one, two, or three dimensions to form filaments, layers, and crystals. Here, we report the atomic structure of a three-dimensional protein cage with a tetrahedral shape, which self-assembles from 12 identical copies of a large (50 kD) designed protein subunit (Fig. 1).

The design reported here has its basis in principles of symmetry, which have played an important role in various strategies for designing protein assemblies. A number of recent studies have reported the creation of symmetric, well-ordered assemblies composed of a few protein molecules (1–4). Designing ordered assemblies of larger size and complexity has been a greater challenge.

Several years ago a symmetry-based idea was put forward for designing a wide range of large, complex materials by combining two distinct oligomeric protein domains, each conferring a symmetric mode of association such as dimerization or trimerization, into a single protein molecule or subunit (5) (Fig. 1). The importance of controlling the relative orientation of the two combined components and their respective symmetry elements was emphasized, and one specific strategy for exerting the necessary geometric control was tested; the two oligomerization domains were connected by a continuous, semirigid α helix. The approach was successful in producing filaments from one designed protein and somewhat polymorphic, cagelike structures from another (6). Recent studies have expanded on the idea by in-

troducing strategic variations for how to connect separate oligomerization motifs (7). So far, however, crystal structures that could help validate symmetric fusion strategies have not been reported.

The protein designed in the present work is a geometrically controlled fusion of two oligomerization domains, a naturally trimeric protein (bromoperoxidase) and a naturally dimeric protein (M1 virus matrix protein), connected by an α -helical linker intended to orient the symmetry axes of the two components so they intersect at an angle half the well-known tetrahedral value of 109.5° (Fig. 1, top). The design is a variation on one of the constructions described earlier (5), which assembled to form structures of heterogeneous size rather than a specific 12-subunit cage as intended (6). Success in obtaining a well-ordered cage with the current design relied critically on making two amino acid changes to the earlier design. After those amino acid changes, homog-

eneous 12-subunit assemblies were obtained nearly exclusively in solution (6), and an x-ray crystal structure was obtained at 3.0 Å resolution (Fig. 1).

The cage is about 160 Å at its largest diameter. Within each subunit, the separate dimeric and trimeric oligomerization domains are connected by a semirigid linker that retains an α -helical conformation, as designed. However, the helical linkers in the 12 subunits exhibit variable amounts of bending and torsion. This, combined with disturbances in some of the M1 matrix protein dimeric interfaces (8), causes the assembled cage to deviate from perfect tetrahedral geometry by about 8 Å overall (6).

The atomic structure reported here validates the symmetry-based fusion approach for designing ordered protein materials of various types, originally dubbed “nanohedra” (5). The results also emphasize the consequences of flexibility between the linked components and the need for attention to this element during the design stage. Symmetry-based strategies promise to advance the design of new biomaterials, from cages to three-dimensional crystals, potentially rivaling designs already achieved by using nucleic acids instead of protein molecules as the assembly blocks (9).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6085/1129/DC1
Materials and Methods
Figs. S1 to S4
Table S1
References (10–17)

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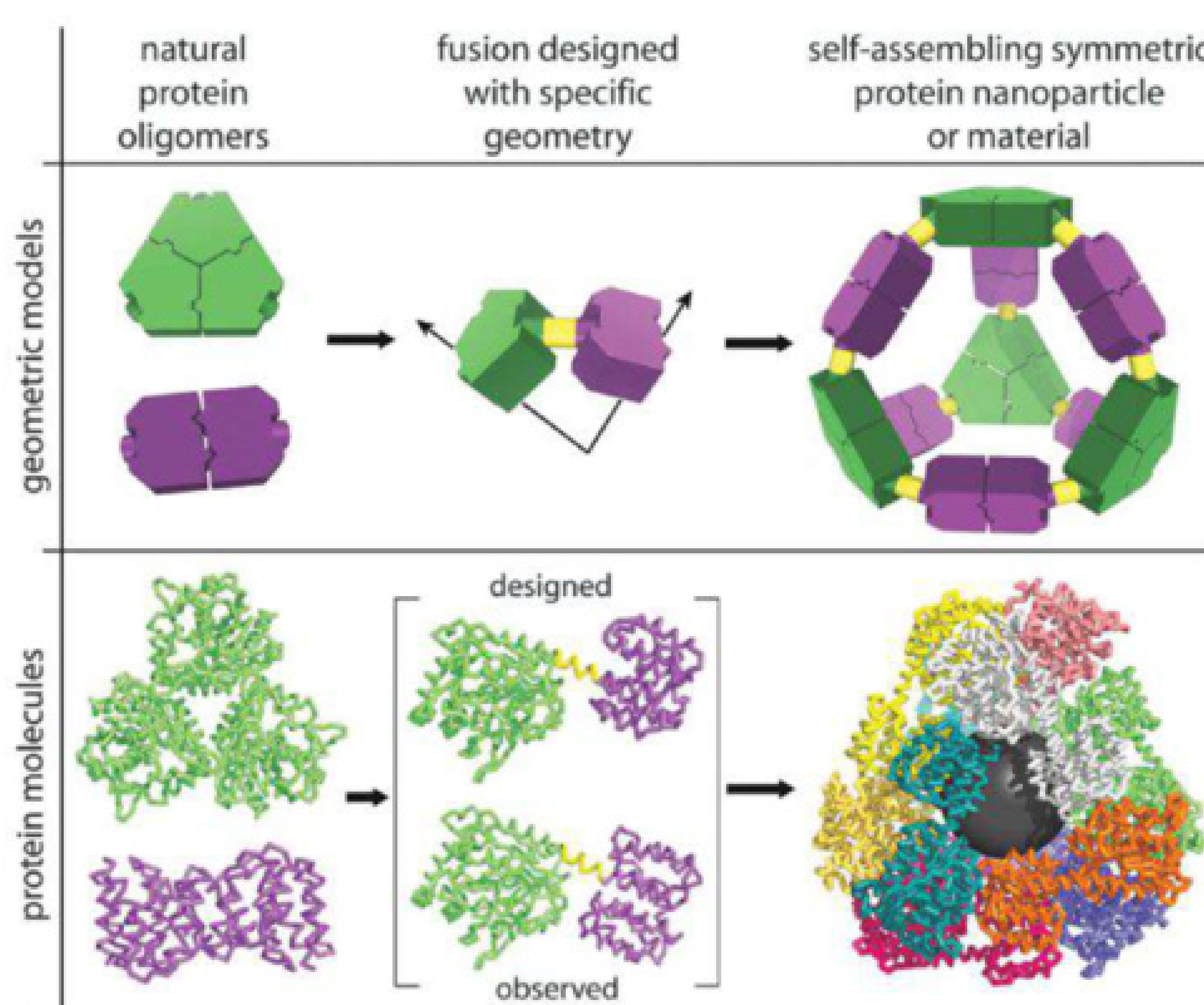


Fig. 1. Atomic structure of a designed protein cage. Geometric models (top) illustrate the design principle of fusing two naturally oligomeric protein domains in a specified geometric arrangement (5). Twelve designed subunits self-assemble to form a tetrahedral cage. (Bottom) The natural protein oligomers (left), and a comparison of a monomer of the designed fusion protein model with its actual crystal structure (middle). The crystal structure of the self-assembling cage (right) confirms the tetrahedral geometry of the designed assembly yet shows deviations from perfect symmetry. The center sphere (50 Å diameter) is shown to emphasize the open nature of the cage.

Quantum Algorithms for Quantum Field Theories

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Quantum field theory reconciles quantum mechanics and special relativity, and plays a central role in many areas of physics. We developed a quantum algorithm to compute relativistic scattering probabilities in a massive quantum field theory with quartic self-interactions (ϕ^4 theory) in spacetime of four and fewer dimensions. Its run time is polynomial in the number of particles, their energy, and the desired precision, and applies at both weak and strong coupling. In the strong-coupling and high-precision regimes, our quantum algorithm achieves exponential speedup over the fastest known classical algorithm.

The question whether quantum field theories can be efficiently simulated by quantum computers was first posed by Feynman three decades ago when he introduced the notion of quantum computers (1). Since then, efficient quantum algorithms for simulating the dynamics of quantum many-body systems have been developed theoretically (2–4) and demonstrated experimentally (5–7). Quantum field theory, which applies quantum mechanics to functions of space and time, presents additional technical challenges, because the number of degrees of freedom per unit volume is formally infinite.

We show that quantum computers can efficiently calculate scattering probabilities in continuum ϕ^4 theory to an arbitrary degree of precision. We have chosen ϕ^4 theory, a scalar theory with quartic self-interactions, because it is among the simplest interacting quantum field theories and thus illustrates essential issues without unnecessary complications. Our work introduces several new techniques, including creation of the initial state by a generalization of adiabatic state preparation and the use of effective field theory to analyze spatial discretization errors.

In complexity theory, the efficiency of an algorithm is judged by how its computational demands scale with the problem size or some other quantity associated with the problem's intrinsic difficulty. An algorithm with polynomial-time asymptotic scaling is considered to be feasible, whereas one with superpolynomial (typically, exponential) scaling is considered infeasible. This classification has proved to be a useful guide in practice.

Traditional calculations of quantum field theory scattering amplitudes rely on perturba-

tion theory—namely, a series expansion in powers of the coupling (the coefficient of the interaction term), which is taken to be small. A powerful and intuitive way of organizing this perturbative expansion is through Feynman diagrams, in which the number of loops is associated with the power of the coupling. A reasonable measure of the computational complexity of perturbative calculations is therefore the number of Feynman diagrams, which is determined by combinatorics and grows factorially with the number of loops and the number of external particles.

If the coupling constant is insufficiently small, the perturbation series does not yield correct results. In ϕ^4 theory, for $D = 2, 3$ spacetime dimensions, by increasing the coupling λ_0 , one eventually reaches a quantum phase transition at some critical coupling λ_c (8–10). In the parameter space near this phase transition, perturbative methods become unreliable; this region is referred to as the strong-coupling regime. There are then no known feasible classical methods for calculating scattering amplitudes, although lattice field theory can be used to obtain static quantities such as mass ratios. Even at weak coupling, the perturbation series is not convergent, although it is asymptotic (11–13). Including higher-order contributions beyond a certain point makes the approximation worse. There is thus a maximum possible precision achievable perturbatively.

We simulate a process in which initially well-separated massive particles with well-defined momenta scatter off each other. The input to our algorithm is a list of the momenta of the incoming particles, and the output is a list of the momenta of the outgoing particles produced by the physical scattering process. At relativistic energies, the number of outgoing particles may differ from the number of incoming particles. In accordance with quantum mechanics, the incoming momenta do not uniquely determine the outgoing momenta, but rather a probability distribution over possible outcomes. Upon repeated runs, our quantum algorithm samples

from this distribution. The asymptotic scaling of the algorithm is given in Eq. 9 and Table 1. The simulated scattering processes closely match experiments in particle accelerators, which are the standard tools to probe quantum field-theoretical effects.

The issue of gauge symmetries in quantum simulation of lattice field theories has been addressed in (14). There is an extensive literature on analog simulation of interacting quantum field theories using ultracold atoms (15–26), trapped ions (27, 28), and Josephson-junction arrays (29). Much work has also been done on analog simulation of special-relativistic quantum mechanical effects such as zitterbewegung and the Klein paradox, as well as general-relativistic quantum effects such as Hawking radiation [for recent reviews, see (30, 31)]. Our work, in contrast to these studies, addresses digital quantum simulation, with explicit consideration of convergence to the continuum limit and efficient preparation of wave packet states for the computation of dynamical quantities such as scattering probabilities. Our analysis includes error estimates of all parts of our algorithm.

Representing fields with qubits. Although quantum field theory is typically expressed in terms of Lagrangians and within the interaction picture, our algorithm is more naturally described in the formalism of Hamiltonians and within the Schrödinger picture. We start by defining a lattice ϕ^4 theory and subsequently address convergence to the continuum theory. (In $D = 4$, the continuum limit is believed to be the free theory. Nonetheless, because the coupling shrinks only logarithmically, scattering processes for particles with small momenta in lattice units are interesting to compute.) Let $\Omega = a\mathbb{Z}_L^d$, that is, an $\hat{L} \times \dots \times \hat{L}$ lattice in $d = D - 1$ spatial dimensions with periodic boundary conditions and lattice spacing a . The number of lattice sites is $V = \hat{L}^d$. For each $\mathbf{x} \in \Omega$, let $\phi(\mathbf{x})$ be a continuous, real degree of freedom—interpreted as the field at $\mathbf{x}$ —and let $\pi(\mathbf{x})$ be the corresponding canonically conjugate variable. In canonical quantization, these degrees of freedom are promoted to Hermitian operators with the commutation relation

$$[\phi(\mathbf{x}), \pi(\mathbf{y})] = ia^{-d}\delta_{\mathbf{x},\mathbf{y}}\mathbb{1} \quad (1)$$

We use units with $\hbar = c = 1$. ϕ^4 theory on the lattice Ω is defined by the Hamiltonian

$$H = \sum_{\mathbf{x} \in \Omega} a^d \left[\frac{1}{2} \pi(\mathbf{x})^2 + \frac{1}{2} (\nabla_a \phi)^2(\mathbf{x}) + \frac{1}{2} m_0^2 \phi(\mathbf{x})^2 + \frac{\lambda_0}{4!} \phi(\mathbf{x})^4 \right] \quad (2)$$

where $\nabla_a \phi$ denotes a discretized derivative (that is, a finite-difference operator) and m_0 is the particle mass of the corresponding noninteracting ($\lambda_0 = 0$) theory.

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We represent the state of the lattice field theory by devoting one register of qubits to store the value of the field at each lattice point. In principle, each $\phi(\mathbf{x})$ is an unbounded continuous variable. To represent the field at a given site with finitely many qubits, we cut off the field at a maximum magnitude $\phi_{\max}$ and discretize it in increments of δ_ϕ . This requires $n_b = O(\log_2(\phi_{\max}/\delta_\phi))$ qubits per site. Note that this field discretization is a separate issue from the spatial discretization via the lattice Ω .

Let $|\psi\rangle$ be any state such that $\langle\psi|H|\psi\rangle \leq E$. The probability distribution over $\phi(\mathbf{x})$ defined by $|\psi\rangle$ (for any $\mathbf{x} \in \Omega$) yields a very low probability (32, 33) for $|\phi(\mathbf{x})|$ to be much larger than $O(E^{1/2})$. Thus, a cutoff $\phi_{\max} = O((VE/a^d m_0^2 \epsilon)^{1/2})$ suffices to ensure fidelity $1 - \epsilon$ to the original state $|\psi\rangle$. One can prove this by bounding $\langle\psi|\phi(\mathbf{x})|\psi\rangle$ and $\langle\psi|\phi^2(\mathbf{x})|\psi\rangle$ as functions of E and applying Chebyshev's inequality (33). To choose δ_ϕ , note that the eigenbasis of $a^d \pi(\mathbf{x})$ is the Fourier transform of the eigenbasis of $\phi(\mathbf{x})$. Hence, discretizing $\phi(\mathbf{x})$ in units of δ_ϕ is equivalent to introducing the cutoff $-\pi_{\max} \leq \pi(\mathbf{x}) \leq \pi_{\max}$, where $\pi_{\max} = 1/(a^d \delta_\phi)$. By bounding the expectations of $\pi(\mathbf{x})$ and $\pi^2(\mathbf{x})$, one finds that it suffices to choose $\pi_{\max} = O((VE/\epsilon a^d)^{1/2})$, and thus $n_b = O(\log_2(VE/m_0 \epsilon))$.

The algorithm. We now turn to the main three tasks of quantum simulation: preparing an initial state, simulating the time evolution, and measuring final observables. We first discuss the simulation of time evolution because it is used in all three tasks. The unitary operator representing time evolution, $\exp(-iHt)$, can be approximated by a quantum circuit of $O((tV)^{1+(1/2k)})$ gates implementing a k th-order Suzuki-Trotter formula (34, 35). For n time steps, the error is $\epsilon \sim n^{-2k}$. The near-linear scaling with t has long been known. The scaling with V is a consequence of the locality of H (33) and appears not to have been noted previously in the quantum algorithms literature.

To simulate scattering, one needs to prepare an initial state of particles in well-separated wave packets. We do so by preparing the vacuum of the $\lambda_0 = 0$ theory, exciting wave packets, and then adiabatically turning on the coupling λ_0 . Let $H^{(0)}$ be the Hamiltonian obtained by setting $\lambda_0 = 0$ in H . $H^{(0)}$ defines an exactly solvable model in which the particles are noninteracting. The vacuum (ground) state $|\text{vac}(0)\rangle$ of $H^{(0)}$ is a multivariate Gaussian wave function in the variables $\{\phi(\mathbf{x})|\mathbf{x} \in \Omega\}$ and can therefore be prepared via the method of Kitaev and Webb (36). The asymptotic scaling of the Kitaev-Webb method is dictated by the computation of the $\mathbf{LDL}^T$ decomposition of the inverse covariance matrix, which can be done classically in $O(V^{2.376})$ time (37, 38).

In analogy with the harmonic oscillator, one can define creation and annihilation operators $a_{\mathbf{p}}$ and $a_{\mathbf{p}}^\dagger$ such that $H^{(0)} = \sum_{\mathbf{p} \in \Gamma} L^{-d} \omega(\mathbf{p}) a_{\mathbf{p}}^\dagger a_{\mathbf{p}} + E^{(0)} \mathbb{1}$, where $\Gamma = (2\pi/\hat{L}a)\mathbb{Z}_L^d$ is the momentum-space lattice corresponding to Ω , $\omega(\mathbf{p}) = [m_0^2 + (4/a^2) \sum_{j=1}^d \sin^2(ap_j/2)]^{1/2}$, and $E^{(0)}$ is an irrele-

vant zero-point energy. The operator $a_{\mathbf{p}}^\dagger$ can be interpreted as creating a (completely delocalized) particle of the noninteracting theory with momentum $\mathbf{p}$ and energy $\omega(\mathbf{p})$.

The (unnormalized) state $\phi(\mathbf{x})|\text{vac}(0)\rangle$ is interpreted as a single particle localized at $\mathbf{x}$ [see, e.g., (39)]. Because $a_{\mathbf{p}}|\text{vac}(0)\rangle = 0$, $\phi(\mathbf{x})|\text{vac}(0)\rangle = a_{\mathbf{x}}^\dagger|\text{vac}(0)\rangle$, where

$$a_{\mathbf{x}}^\dagger = \sum_{\mathbf{p} \in \Gamma} L^{-d} \exp(-i\mathbf{p} \cdot \mathbf{x}) \left[\frac{1}{2\omega(\mathbf{p})} \right]^{1/2} a_{\mathbf{p}}^\dagger \quad (3)$$

The operator

$$a_\psi^\dagger = \eta(\psi) \sum_{\mathbf{x} \in \Omega} a^d \psi(\mathbf{x}) a_{\mathbf{x}}^\dagger \quad (4)$$

creates a wave packet with position-space wave function ψ ; $\eta(\psi)$ is a normalization constant, chosen so that $[a_\psi, a_\psi^\dagger] = 1$. $a_\psi^\dagger$ is not unitary, so it cannot be directly implemented by a quantum circuit. Instead, we introduce an ancillary qubit and let

$$H_\psi = a_\psi^\dagger \otimes |1\rangle\langle 0| + a_\psi \otimes |0\rangle\langle 1| \quad (5)$$

One can verify that $\exp(-iH_\psi \pi/2)|\text{vac}(0)\rangle|0\rangle = -ia_\psi^\dagger|\text{vac}(0)\rangle|1\rangle$. Using a high-order Suzuki-Trotter formula (34, 35), we can construct an efficient quantum circuit approximating the unitary transformation $\exp(-iH_\psi \pi/2)$. Applied to $|\text{vac}(0)\rangle$, this circuit yields the desired state up to an irrelevant global phase and an unentangled ancillary qubit, which can be discarded. We repeat this process for each incoming particle desired.

Because we wish to create localized wave packets, we choose $\psi(\mathbf{x})$ to have bounded support. Expanding $a_\psi^\dagger$ in terms of the operators ϕ and π yields an expression of the form $a_\psi^\dagger = \sum_{\mathbf{x} \in \Omega} [f(\mathbf{x})\phi(\mathbf{x}) + g(\mathbf{x})\pi(\mathbf{x})]$, where $f(\mathbf{x})$ and $g(\mathbf{x})$ are exponentially decaying with characteristic length scale $1/m_0$ outside the support of ψ . Thus, a_ψ and $a_\psi^\dagger$ can be exponentially well approximated by linear combinations of the operators ϕ and π on a local region of space, and the complexity of simulating $\exp(-iH_\psi \pi/2)$ does not scale with the volume V . Furthermore, provided the initial wave packets are separated by a distance δ that is large relative to $1/m_0$, the preparation of each additional wave packet leaves the existing wave packets almost perfectly undisturbed [with errors on the order of $\exp(-m_0 \delta)$].

At this point, we have finished constructing wave packets of the noninteracting theory. We next use a Suzuki-Trotter formula to construct a quantum circuit simulating the unitary transformation induced by a time-dependent Hamiltonian in which the coupling constant is gradually increased from zero to its final value, λ_0 , over time τ . A perfectly adiabatic turn-on would ensure that no stray particles are created during this process, provided particle creation costs energy, that is, the particles have nonzero mass. Imperfect adiabaticity causes errors, namely, particle creation from the vacuum and the splitting of one

particle into three. The probabilities of such errors depend significantly on the particle mass.

In the $D = 2, 3$ interacting theory, with fixed m_0 and sufficiently large λ_0 , the mass vanishes. This marks the location of the $\phi \rightarrow -\phi$ symmetry-breaking transition. Here, we restrict our attention to simulations within the symmetric phase, but we do consider systems arbitrarily close to the phase transition, because these should be particularly hard to simulate classically. In this regime, the mass exhibits known power-law behavior.

As Eq. 4 shows, wave packets are not eigenstates of $H^{(0)}$. During the adiabatic turn-on, the different eigenstates acquire different dynamical phases. Thus, as the wave packet time evolves, it propagates and broadens. This behavior is undesirable in our simulation, because we do not wish the particles to collide and scatter before the coupling reaches its final value. We therefore introduce backward time evolutions governed by time-independent Hamiltonians into the adiabatic state-preparation process to undo the dynamical phases. Specifically, let $H(s)$ parameterize the adiabatic time evolution, with $H(0) = H^{(0)}$ and $H(1) = H$. We divide the adiabatic preparation into J steps, with U_j denoting the unitary time evolution induced by the time-dependent Hamiltonian linearly interpolating between $H((j-1)/J)$ and $H(j/J)$ over a period of τ/J . Let M_j consist of backward, forward, and backward evolutions, namely,

$$M_j = \exp \left[iH \left(\frac{j+1}{J} \right) \frac{\tau}{2J} \right] U_j \exp \left[iH \left(\frac{j}{J} \right) \frac{\tau}{2J} \right] \quad (6)$$

Our full state-preparation process is $\prod_{j=1}^J M_j$. The dynamical phases are undone, converging to zero as $J \rightarrow \infty$, while the adiabatic change of eigenbasis is undisturbed (33).

What is the complexity of adiabatic state preparation? At weak coupling for $d = 1, 2$, a wave packet travels a distance $O(\tau/J^2)$ as the coupling is turned on; therefore, choosing $J = O(\tau^{1/2})$ ensures that this distance is a suitably small constant. Adiabatic transitions occur with probability $O((J/\tau\gamma^2)^2)$, where γ is the energy gap of the transition; hence, the state preparation error is $\epsilon = O(1/\tau)$. The number of quantum gates needed to simulate this process is $O((\tau V)^{1+o(1)}) = O((\tau a^{-d})^{1+o(1)})$ in a fixed physical volume; setting $\tau = O(\epsilon^{-1})$ and $a = O(\epsilon^{1/2})$ (see below), we conclude that the complexity scales as $O((1/\epsilon)^{1+(d/2)})$. For $d = 3$, the scaling with ϵ is further enhanced because the perturbative corrections to the physical mass are highly sensitive to the value of a . Complexity scalings at strong coupling can be obtained by analogous reasoning (33).

After the system has evolved for a period in which scattering occurs, measurement can be performed in one of two ways. The interaction can be adiabatically turned off through the time-reversed version of the turn-on described above; in the free theory, we can then measure the number operators of the momentum modes, using the

method of phase estimation (40), that is, by simulating $\exp(iL^{-d}a_p^\dagger a_p t)$ for various values of t and Fourier-transforming the results. Alternatively, one can divide space into small regions and measure the total energy and momentum operators in each region using phase estimation. This is a reasonable idealization of real detectors and can yield information about bound-state energies.

Discretization errors. Having described how to simulate scattering processes in a discretized quantum field theory, we now consider discretization errors. To analyze these errors, we use methods of effective field theory (EFT), a well-developed formalism underlying our modern understanding of quantum field theory.

In its regime of validity, typically below a particular energy scale, an EFT reproduces the behavior of the full (that is, fundamental) theory under consideration: It can be regarded as the low-energy limit of that theory. An EFT for a full theory is thus somewhat analogous to a Taylor series for a function. It involves an expansion in some suitable small parameter, so that, although it consists of infinitely many terms, higher-order terms are increasingly suppressed. Thus, the series can be truncated, with corresponding finite and controllable errors.

We apply this framework to analyze the effect of discretizing the spatial dimensions of the continuum ϕ^4 quantum field theory. The discretized Lagrangian can be thought of as the leading contribution (denoted by $\mathcal{L}^{(0)}$) to an EFT. From the leading operators left out, we can thus infer the scaling of the error associated with a nonzero lattice spacing a .

The full (untruncated) effective Lagrangian will have every coupling respecting the $\phi \rightarrow -\phi$ symmetry and so will take the form

$$\mathcal{L}_{\text{eff}} = \mathcal{L}^{(0)} + \frac{c}{6!} \phi^6 + c' \phi^3 \partial^2 \phi + \frac{c''}{8!} \phi^8 + \dots \quad (7)$$

This can be simplified. First, the chain rule and integration by parts can be used to write any operator with two derivatives acting on different fields in the form $\phi^n \partial^2 \phi$. For example, $\phi^2 \partial_\mu \phi \partial^\mu \phi = (1/3) \partial_\mu (\phi^3) \partial^\mu \phi \rightarrow -(1/3) \phi^3 \partial^2 \phi$. Such an operator can then be simplified via the equation of motion (41, 42). If this were the equation of motion of the continuum theory, any derivative operator would then be completely eliminated. In the discretized theory, however, the equation of motion is modified and there are residual, Lorentz-violating operators. In fact, because the difference operators in the discretized theory are only approximately equal to the derivatives in the continuum theory, the simplest Lorentz-violating operators are induced purely by discretization.

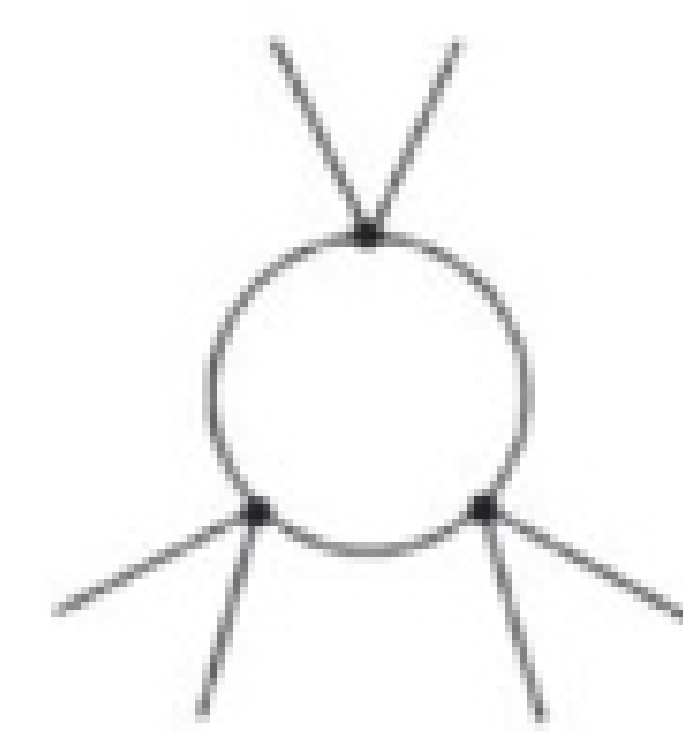
In units where $\hbar = c = 1$, all quantities have units of some power of mass. The mass dimensions (denoted by square brackets) of the

field and coupling in $D = d + 1$ spacetime dimensions are $[\phi] = (D - 2)/2$ and $[\lambda] = 4 - D$, implying that

$$[c] = 6 - 2D, [c''] = 8 - 3D \quad (8)$$

In $D = 4$ dimensions, $[c] = -2$ and $[c''] = -4$, and the only pertinent dimensionful parameter is the lattice spacing; therefore, $c \sim a^2$ and $c'' \sim a^4$. Thus, of the operators not included in the Lagrangian $\mathcal{L}^{(0)}$, ϕ^6 is more significant than ϕ^{2n} , for $n > 3$.

In $D = 2, 3$, the scaling of the coefficients with a is somewhat less obvious, because now the coupling λ provides another dimensionful parameter. To obtain the scaling of c , one should consider the Feynman diagram that generates the corresponding operator. This involves three ϕ^4 vertices, so



$$\sim \lambda^3 a^{6-D}$$

(Other diagrams involve higher powers of λ ; hence their contributions to the operator are suppressed by higher powers of a .) Likewise, the coefficient of ϕ^8 will scale as $\lambda^4 a^{8-D}$, which means that it is suppressed by a^2 relative to the coefficient of ϕ^6 .

The EFT thus consists of three different classes of operators: operators of the form ϕ^{2n} , Lorentz-violating operators arising solely from discretization effects, and Lorentz-violating operators resulting from discretization and quantum effects. These are shown with the scaling of their coefficients in Table 2, which reveals that the dominant discretization errors scale as a^2 in $D = 2, 3, 4$. (In $D = 2, 3$, errors of type II dominate. In $D = 4$, errors of types I and II each scale as a^2 .)

At strong coupling, the operators and their scaling remain the same at the scale of the matching of the full theory onto the EFT, although the explicit coefficients are no longer

Table 1. The asymptotic scaling of the number of quantum gates needed to simulate scattering in the strong-coupling regime in $d = 1, 2$ spatial dimensions is polynomial in p (the momentum of the incoming pair of particles), $\lambda_c - \lambda_0$ (the distance from the phase transition), and n_{out} (the maximum kinematically allowed number of outgoing particles). The notation $f(n) = \tilde{O}(g(n))$ means $f(n) = O(g(n) \log^c(n))$ for some constant c .

	$\lambda_c - \lambda_0$	p	n_{out}
$d = 1$	$\left(\frac{1}{\lambda_c - \lambda_0}\right)^{9+o(1)}$	$p^{4+o(1)}$	$\tilde{O}(n_{\text{out}}^5)$
$d = 2$	$\left(\frac{1}{\lambda_c - \lambda_0}\right)^{6.3+o(1)}$	$p^{6+o(1)}$	$\tilde{O}(n_{\text{out}}^{7.128})$

calculable. However, the running of the coefficients down to lower energies is determined by their so-called anomalous dimensions, which depend on the coupling strength. These anomalous dimensions modify the scaling; at weak coupling the modification is small, but at strong coupling it could be larger. (Still, the scaling will remain polynomial.)

Concluding remarks. The most computationally costly part of the algorithm is either adiabatic state preparation or preparation of the free vacuum, depending on the asymptotic scaling considered and the number of spatial dimensions (33). Because $a \sim \epsilon^{1/2}$, preparation of the free vacuum has complexity $\sim V^{2.376} \sim a^{-2.376d} \sim \epsilon^{2.376d/2}$. The number of gates, G_{weak} , needed to simulate weakly coupled, $(d + 1)$ -dimensional ϕ^4 theory with accuracy $\pm \epsilon$ scales as follows:

$$G_{\text{weak}} \sim \begin{cases} \left(\frac{1}{\epsilon}\right)^{1.5+o(1)}, & d = 1 \\ \left(\frac{1}{\epsilon}\right)^{2.376+o(1)}, & d = 2 \\ \left(\frac{1}{\epsilon}\right)^{5.5+o(1)}, & d = 3 \end{cases} \quad (9)$$

The notation $f(n) = o(g(n))$ means $\lim_{n \rightarrow \infty} f(n)/g(n) = 0$. The asymptotic scaling of the number of gates used to simulate the strongly coupled theory is summarized in Table 1. The minimum number of (perfect) qubits required for a nontrivial simulation in $D = 2$ is estimated—necessarily crudely—to be on the order of 1000 to 10,000, corresponding to ϵ ranging from 10% to 1% (33).

We have shown that quantum computers can efficiently calculate scattering probabilities in ϕ^4 theory to arbitrary precision at both weak and strong coupling. Known classical algorithms take exponential time to do this in the strong-coupling and high-precision regimes. In addition to establishing a new exponential quantum speedup, our results lead the way toward a quantum algorithm for simulating the Standard Model of particle physics, which has new features (not exhibited by ϕ^4 theory) such as chiral fermions and gauge interactions. Such an algorithm would establish that, except for quantum-gravity effects, the standard quantum circuit model suffices to capture completely the computational power of our universe.

Table 2. Effective field theory operators fall into three classes. The general operator in each class is shown, with the canonical scaling of its coefficient in D spacetime dimensions. $\partial_x^{2l} = \sum_{i=1}^d \partial_i^{2l}$.

Class	Operators	Scaling of coupling
I	$\phi^{2n} (n \geq 3)$	$\lambda^n a^{2n-D}$
II	$\phi \partial_x^{2l} \phi (l \geq 2)$	a^{2l-2}
III	$\phi^{2j+1} \partial_x^{2l} \phi (j \geq 1, l \geq 2)$	$\lambda^{j+1} a^{2j+2l+2-D}$

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Supplementary Materials

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 Texts S1 to S7
 Figs. S1 and S2
 References (43–47)

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REPORTS

The Detection and Characterization of a Nontransiting Planet by Transit Timing Variations

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The Kepler mission is monitoring the brightness of ~150,000 stars, searching for evidence of planetary transits. As part of the Hunt for Exomoons with Kepler (HEK) project, we report a planetary system with two confirmed planets and one candidate planet discovered with the publicly available data for KOI-872. Planet b transits the host star with a period $P_b = 33.6$ days and exhibits large transit timing variations indicative of a perturber. Dynamical modeling uniquely detects an outer nontransiting planet c near the 5:3 resonance ($P_c = 57.0$ days) with a mass 0.37 times that of Jupiter. Transits of a third planetary candidate are also found: a 1.7–Earth radius super-Earth with a 6.8-day period. Our analysis indicates a system with nearly coplanar and circular orbits, reminiscent of the orderly arrangement within the solar system.

If a planet's orbit is viewed nearly edge-on, the planet may transit over the disk of its host star and periodically block a small fraction of the starlight. The planet's presence is then revealed by a small and repetitive decrease of the host star's brightness during transits. The transit light curve is characterized by the time of transit minimum τ , the transit depth δ , the total duration T_{14} , and the partial duration T_{23} (*1*). A precise measurement of these terms allows an observer to infer the physical properties of the system, such as the radius ratio $p = R_p/R_*$, transit impact pa-

rameter b_p , and scaled semimajor axis a_p/R_* , where R_* is the star's physical radius and the P subscript denotes "planet."

For a planet following a strictly Keplerian orbit, the spacing, timing, and other properties of the transit light curve should be unchanging in time. Several effects, however, can produce deviations from the Keplerian case so that the spacing of τ is not strictly periodic and/or T_{14} varies from transit to transit. Such changes are known as transit timing variations (TTVs) and transit duration variations (TDVs), respectively. TTVs are

particularly sensitive to gravitational perturbations from additional planets orbiting the host star (2–4) and distant large moons orbiting the transiting planet (5–7).

As part of the Hunt for Exomoons with Kepler (HEK) project (8), we analyzed the publicly available Kepler data up to Quarter 6 (Q6; released on 7 January 2012). At the time of writing, the 33.6-day-period planetary candidate Kepler-object-of-interest 872.01 (KOI-872.01) is the only known candidate in the system (9). The candidate was identified through HEK's target selection procedure as a high-priority object because of the presence of visual transit anomalies and the dynamical capacity to host a moon.

We detrended the raw Kepler photometry of KOI-872, covering 15 transits, using a harmonic filter and an exponential decay ramp correction (10). We tested several models to explain the photometry using the multimodal nested sampling algorithm MULTINEST (11, 12), designed to compute the Bayesian evidence for each model.

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The favored model was found to be that of a planet undergoing TTVs (each transit has a unique τ but common parameters for all other terms; model $\mathcal{M}_7$). This model is preferred over that of a planet on a linear ephemeris, $\mathcal{M}_P$, at a confidence of $\leq 43.7\sigma$ (Fig. 1).

We computed TTVs relative to the linear ephemeris derived from $\mathcal{M}_P$ ($P_b = 33.60134 \pm 0.00021$ days and $\tau_0 = 2455053.2826 \pm 0.0014$ BJD_{UTC}, where BJD_{UTC} is barycentric Julian date in coordinated universal time). The results (Fig. 2) indicate that KOI-872.01 exhibits large (2 hours) and complex TTVs, with a dominant period of ≈ 5.6 transit cycles (≈ 190 days). These are among the largest TTVs ever detected. A model including TDVs is disfavored relative to the TTV-only model at a confidence of 17.5σ .

Because a moon should induce TDVs and TTVs, the lack of TDVs does not favor a moon hypothesis. Further, Q4-Q6 photometry shows complex stellar activity, which may be responsible for the initially identified visual anomalies. In support of these arguments, we found a planet-with-moon model ($\mathcal{M}_M$) inadequate to explain the measured TTVs (Fig. 2C).

A distant stellar companion or secular/resonant perturbations from a planetary companion also cannot explain KOI-872.01's TTVs, because these effects would produce sinusoidal patterns with a very long period (13). Moreover, other known TTV effects, such as parallax effects (14), the Applegate effect (15), and stellar proper motion (16), can be ruled out because they are unable to produce such a large TTV amplitude.

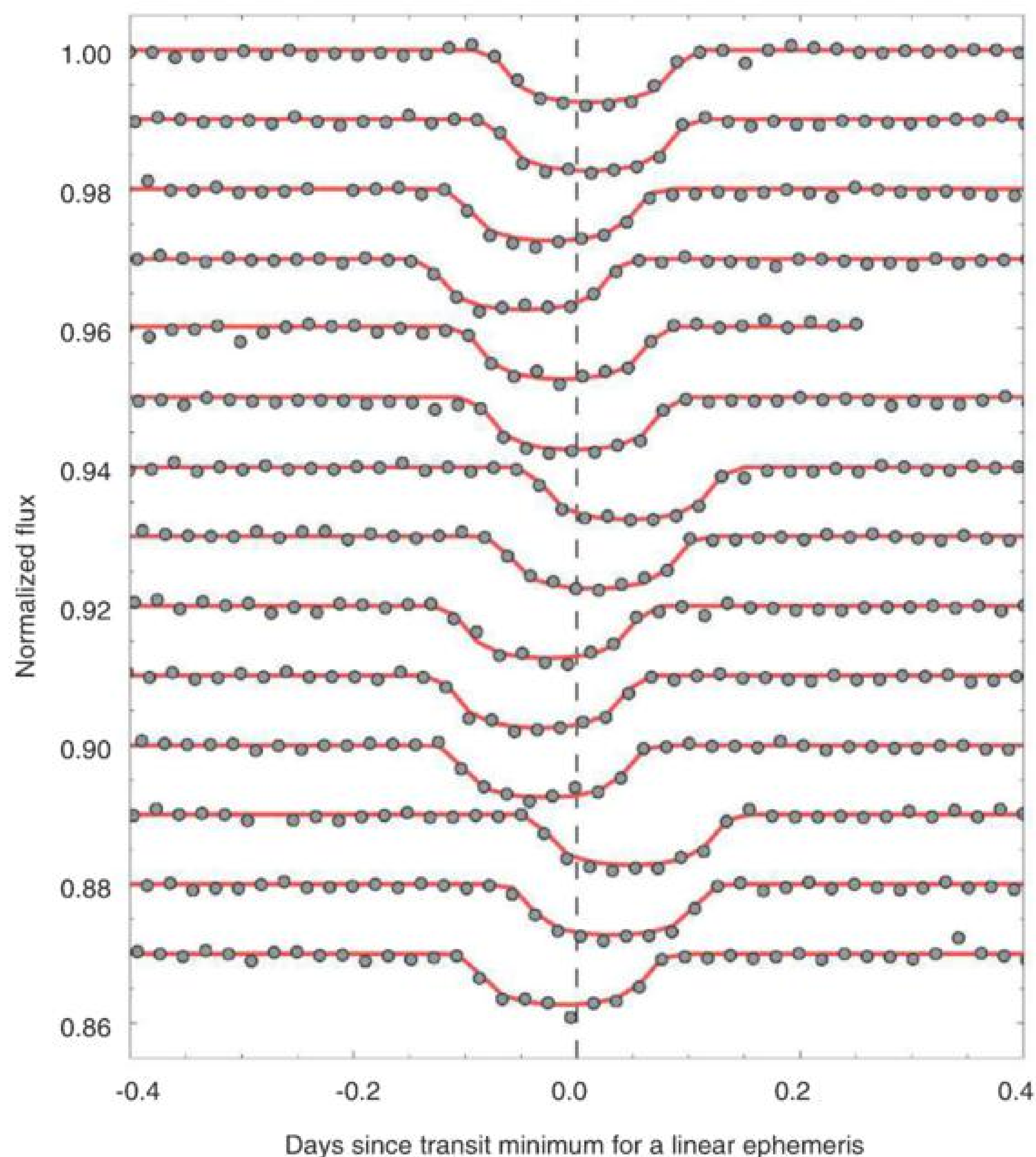


Fig. 1. Maximum-likelihood transit model (red line) overlaid with the long-cadence Kepler offsetted data for KOI-872b. The large TTVs are evident visually from the light curve. The ramp-affected transit is excluded here (see fig. S2).

Fig. 2. Transit timings variations. The measured TTVs (from model $\mathcal{M}_7$) and TDVs (from model $\mathcal{M}_P$) and their uncertainties are indicated in (A) and (C). (A) Calculated values for s1 (red line). The TDVs of s1 are consistent with the measured, flat TDV profile. (C) s2 (blue line) and our best Moon model (green line). Although s2 also fits the measured TTVs relatively well, the strong TDV trend in (C) is inconsistent with the measurements. Panels (B) and (D) show our predictions for s1 and s2.

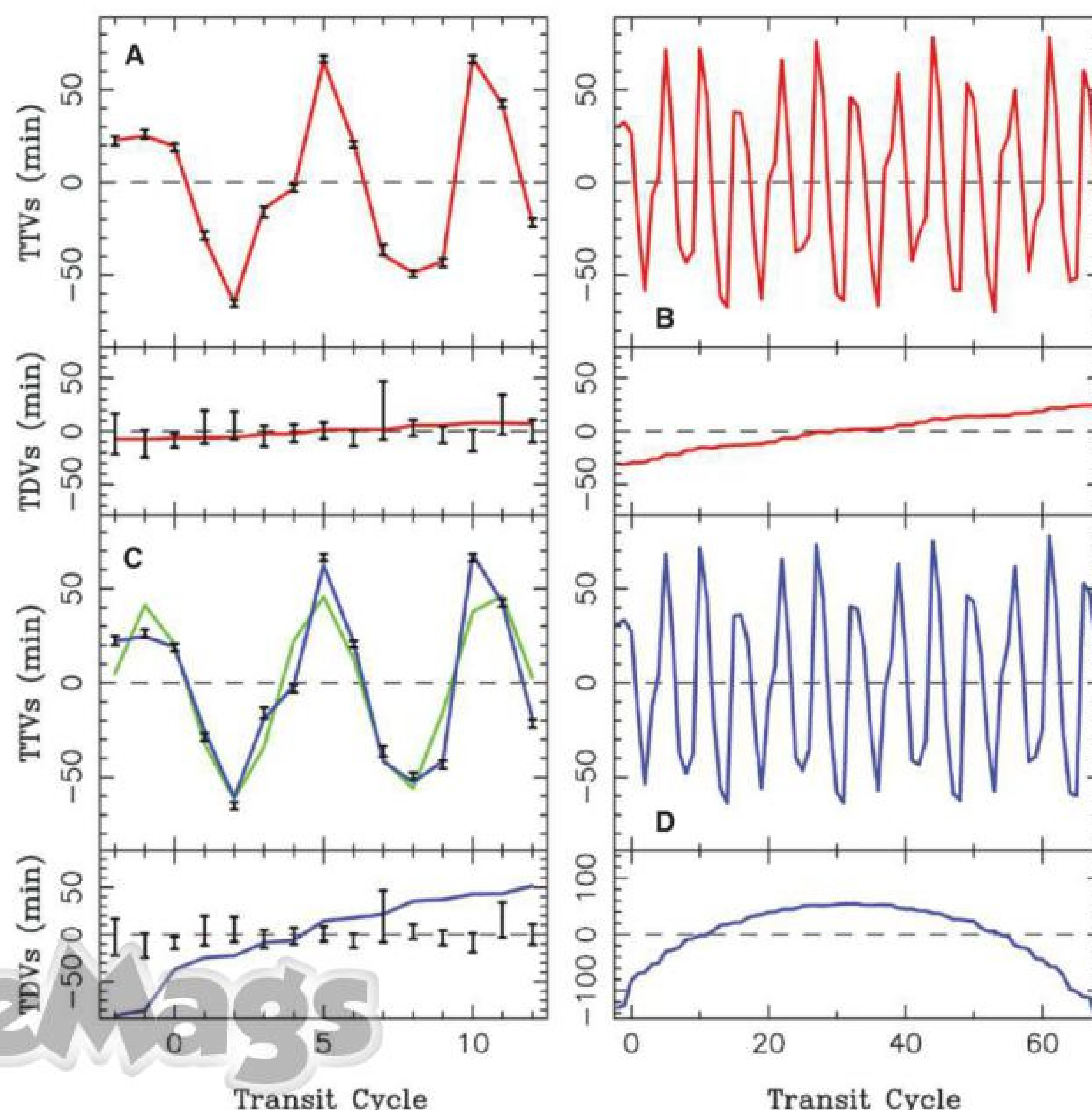


Table 1. KOI-872 system parameters. KOI-872b parameters were computed from the weighted posteriors of a model accounting for TTVs, using MULTINEST. Parameters fitted in the transit model are quoted as the median of the marginalized posteriors with $\pm 34.13\%$ credible intervals. Instrumental terms and times of transit minimum may be found in table S3. KOI-872c parameters were computed from the fit to KOI-872b's TTVs. Parameters fitted in the TTV model are quoted as maximum likelihood with $\pm 34.13\%$ uncertainties computed using the $\Delta\chi^2 = 1$ method described in (27), where the TTV errors have been rescaled such that $\chi^2_{\text{reduced}} = 1$. The 99% confidence areas from the TTV fit alone are shown in fig. S10. The measured TDVs do not offer a meaningful constraint, because the parameter sets near s1 that fit the TTVs also fit the TDVs. Orbital longitudes of KOI-872c are relative to the transit reference system on 2455053.2826 BJD_{UTC}. KOI-872.03 parameters were computed from an MCMC run. Moon mass constraint (3σ limit) was derived from model $\mathcal{M}_{MT2,RO}$ (10).

	KOI-872b	KOI-872c	KOI-872.03
τ_0 (BJD _{UTC})	2455053.2826 $^{+0.0013}_{-0.0014}$	—	2455255.2603 $^{+0.0032}_{-0.0031}$
P_P (days)	33.60134 $^{+0.00021}_{-0.00020}$	57.011 $^{+0.051}_{-0.061}$	6.76671 $^{+0.00013}_{-0.00012}$
R_P/R_*	0.0887 $^{+0.0010}_{-0.0012}$	—	0.01656 $^{+0.00079}_{-0.00082}$
b_P	0.757 $^{+0.022}_{-0.027}$	2.9 $^{+1.1}_{-1.8}$	0.39 $^{+0.19}_{-0.12}$
a_P/R_*	45.1 $^{+2.1}_{-1.7}$	64.1 $^{+2.9}_{-2.5}$	15.58 $^{+0.51}_{-0.49}$
i_P (°)	89.038 $^{+0.075}_{-0.067}$	87.4 $^{+1.6}_{-1.0}$	88.55 $^{+0.49}_{-0.69}$
a_P (AU)	0.1968 $^{+0.0029}_{-0.0028}$	0.2799 $^{+0.0041}_{-0.0040}$	0.0679 $^{+0.0035}_{-0.0035}$
e_P	0.01 $^{+0.01}_{-0.01}$	0.0146 $^{+0.0034}_{-0.0036}$	0 (assumed)
Ω_P (°)	270	298 $^{+37}_{-36}$	—
ϖ_P (°)	—	330.0 $^{+11.6}_{-9.2}$	—
λ_P (°)	0	338.2 $^{+1.2}_{-1.4}$	—
M_P/M_*	$<6.4 \times 10^{-3}$	3.97 $^{+0.15}_{-0.11} \times 10^{-4}$	—
M_P (M_J)	<6	0.376 $^{+0.021}_{-0.019}$	—
R_P (R_J)	0.808 $^{+0.042}_{-0.043}$	—	0.1510 $^{+0.0094}_{-0.0098}$
ρ_P (kg m $^{-3}$)	<14000	—	—
T_{eq} (K)	543 $^{+16}_{-16}$	455 $^{+13}_{-14}$	924 $^{+24}_{-23}$
M_{moon}/M_P	<0.021	—	—
KOI-872			
ρ_* (kg m $^{-3}$)	1530 $^{+220}_{-170}$		
M_* ($M_\odot$)	0.902 $^{+0.040}_{-0.038}$		
R_* ($R_\odot$)	0.938 $^{+0.038}_{-0.039}$		
$\log g_*$	4.447 $^{+0.040}_{-0.035}$		
T_{eff} (K)	5155 $\pm$ 105		
L_* ($L_\odot$)	0.556 $^{+0.078}_{-0.070}$		
M_V	5.60 $^{+0.17}_{-0.17}$		
Age (Gyr)	9.7 $^{+3.7}_{-3.5}$		
Distance (pc)	855 $^{+68}_{-65}$		
(M/H)	0.41 $\pm$ 0.10		

We applied the TTV inversion method described in (17) to test whether the observed TTVs are consistent with short-period perturbations from a planetary companion and whether a unique set of parameters can be derived to describe the physical and orbital properties of that companion. The short-period perturbations are small variations around the mean Keplerian orbit of a planet with characteristic periods comparable to the planet's orbital period. The inversion method is based on perturbation theory, which greatly speeds up the computation of the timing and duration of individual transits (18).

We tested orbits with periods between 1 day and 10 years, including the cases of highly eccentric and/or retrograde planets (19). The identified solutions were fine tuned using a precise N -body integrator. We used the downhill simplex method to search for the minimum of $\chi^2 = \sum_{j=-2}^{n-3} (\delta t_{O,j} - \delta t_{C,j})^2 / \sigma_j^2$, where $n = 15$ is the number of transits, $\delta t_{O,j}$ and $\delta t_{C,j}$ are the observed and calculated TTVs, and σ_j is the un-

certainty of $\delta t_{O,j}$. Local minima in χ^2 were tested for physical plausibility, including a stability test where the orbits were tracked over 10^9 years with a symplectic integrator (20).

All except two solutions can be ruled out because they are either dynamically unstable or have $\chi^2_{\text{min}} > 60$. The best-fit solution (hereafter s1) fits the data extremely well: $\chi^2_{\text{min},s1} = 3.4$ for $n - m = 15 - 7 = 8$ degrees of freedom, where $m = 7$ parameters of the perturbing planet are the mass ratio M_c/M_* , period P_c , eccentricity e_c , inclination I_c , nodal longitude Ω_c , pericenter longitude ϖ_c , and mean longitude λ_c . The inclination I is defined relative to the reference plane that is 90° tilted to the sky plane and rotated so that $\Omega_b = 270^\circ$ (all longitudes are measured relative to the line of sight; hereafter, transit reference system). The orbital inclination of KOI-872.01 relative to the transit plane is $I_b = 0.96^\circ$, as determined from the transit fit.

The second best-fit solution (s2) has $\chi^2_{\text{min},s2} = 20.3$. It corresponds to a planet with $M_c \approx 1.8 \times$

$10^{-3} M_*$, $P_c \approx 81.7$ days (i.e., $P_c/P_b = 2.43$, just inside the 5:2 resonance), $e_c \approx 0.03$, and $I_c \approx 10^\circ$. s2 can be ruled out because the goodness-of-fit for the TTVs is significantly worse at $\Delta\chi^2 = 16.9$; also, as I_c is relatively large, s2 implies strong TDVs that are inconsistent with the observations (Fig. 2C) (21).

Therefore, the transit variations of KOI-872.01 can only be fit by s1. This was by no means expected or guaranteed because the short-period TTVs produced by the interacting planets represent only a very specific subset of astrophysical signals. This can be demonstrated by scrambling the TTV data points and applying the TTV inversion method to the scrambled data. We were unable to find a plausible planet solution for any of the attempted (thousand) trials with the scrambled data. This is a strong indication that KOI-872 is a real system of at least two planets (22) (Table 1).

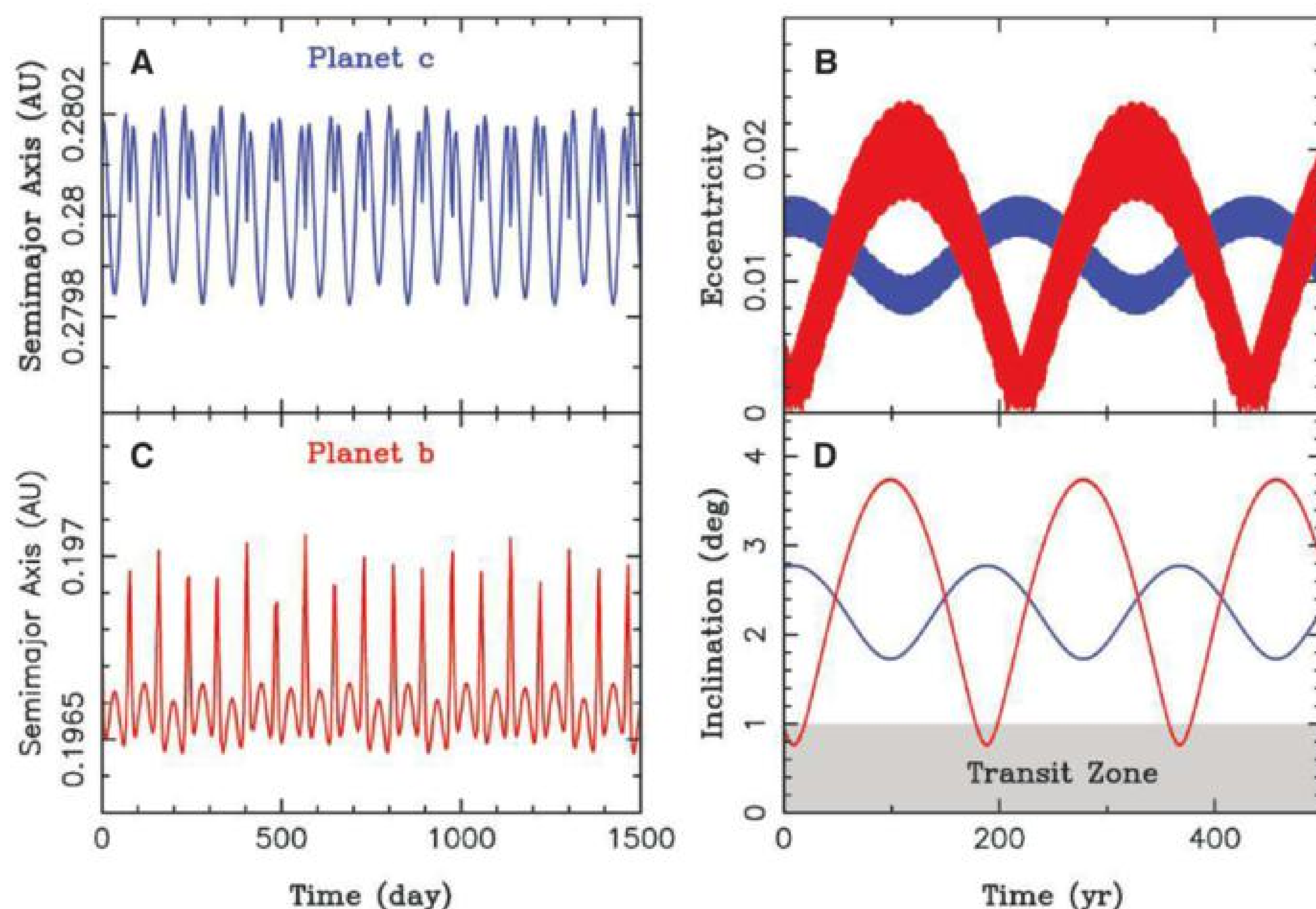
The scaled mass of KOI-872.02 inferred from TTVs of KOI-872.01 is $M_c/M_* = 3.97 \times 10^{-4}$. With $M_* \approx 0.9 M_\odot$, obtained from spectroscopy, this gives $M_c \approx 0.37$ Jupiter mass (M_J), or ~ 1.3 Saturn masses. The mass of KOI-872.01, M_b , cannot be constrained from TTVs because the short-period TTVs are practically independent of M_b (3, 18). The stability requirements imply that $M_b < 6 M_J$, because the orbits are dynamically unstable with a more massive transiting body. This mass limit, along with our vetting analysis (10), confirms KOI-872.01 and the perturber as being genuine planets, henceforth referred to KOI-872b (corresponding to KOI-872.01) and KOI-872c.

The period ratio $P_c/P_b = 1.697$ indicates that the two planets are just outside the 5:3 resonance. This may be a relatively common configuration, probably related to the radial migration of planets in the protoplanetary nebula (23). The resonant angle, $5\lambda_c - 3\lambda_b - 2\varpi_c$, circulates in the retrograde sense with the period of ≈ 2 years. The dominant TTV period (≈ 5.6 transit cycles; Fig. 2) comes from the relatively distant 2:1 and 3:2 terms (periods ≈ 190 years).

The orbital eccentricities are $e_b < 0.02$ and $e_c \approx 0.015$. The nearly circular orbits probably indicate that the two planets formed at, or migrated to, their present orbits without suffering any strong dynamical instability. The two planets also exhibit nearly but not exactly coplanar orbits. The orbital inclination of KOI-872c with respect to the transit plane is $I_c < 4.5^\circ$ (99% confidence interval), with the best TTV fit indicating $I_c = 2.6^\circ$. The best-fit inclination value and $\Omega_c \approx 298^\circ$ obtained from the TTVs suggest that the planet just avoids transiting. A search for the transits of KOI-872c indeed yielded no events, implying that $I_c > 1^\circ$.

Although no transits of KOI-872c were detected, we did detect a 9σ transit signal on a short period of 6.8 days. The transit corresponds to a 1.7-Earth radius super-Earth, which we refer to as KOI-872.03. Because of the probable low-mass nature of this body, the expected

Fig. 3. Orbital evolution. Figure shows the semimajor axis (A and C), eccentricity (B), and inclination (D) for the best-fit solution. The orbital elements of KOI-872b and KOI-872c are shown in red and blue, respectively. The inclination in (D) is defined relative to the transit plane ($i = 0^\circ$ would correspond to a transit across the middle of the host star's disk). An approximate transit zone for $\Omega = 270^\circ$ is shaded. Ω_b and Ω_c are locked near 270° , because the invariant plane of planets is tilted to the transit plane. KOI-872.03 is omitted here because it has a negligible effect on the dynamical evolution of KOI-872b and KOI-872c.



TTVs induced on KOI-872b would be ~ 1 s in amplitude, too small to detect with our data. Further, the TTVs of KOI-872.03 itself are estimated to be ~ 10 s, also undetectable. Without TTVs, we are unable to confirm that KOI-872.03 orbits the same star as KOI-872b and KOI-872c, as opposed to a blended background star. However, our analysis finds that KOI-872.03's light curve derived stellar density is consistent with that of KOI-872b for both planets on near-circular orbits.

We predict that the radial velocity (RV) measurements of KOI-872 should reveal at least two basic periods. The RV term with a 57.0-day period, corresponding to that of KOI-872c, should have $K_1 \approx 20 \text{ m s}^{-1}$ half-amplitude. The amplitude of the 33.6-day-period term, corresponding to that of KOI-872b, is uncertain because we do not have a good constraint on M_b . The 6.8-day term, corresponding to that of KOI-872.03, will be difficult to detect because $K_3 \sim 1$ to 2 m s^{-1} (assuming Earth-like density).

To gain insights into the system's dynamical behavior, we numerically integrated the planetary orbits, starting from the best-fit solution (Fig. 3). The semimajor axis of KOI-872b experiences short-period variations, related to the TTVs, with an amplitude of $\approx 5 \times 10^{-4}$ astronomical units (AU). The eccentricities undergo anticorrelated oscillations, as dictated by the angular momentum conservation, around means $e_b = 0.013$ and $e_c = 0.012$, with a period of ≈ 200 years (corresponding to ϖ_b 's precession period; ϖ_c precesses much more slowly). The anticorrelated oscillations of inclinations relative to the transit plane are a geometrical effect resulting from the shared precession of Ω_b and Ω_c (≈ 200 -year period). The inclinations relative to the invariant plane are nearly constant, with the mutual inclination $\approx 1^\circ$. If I_b relative to the transit plane increases in the next

decades (Fig. 3D), KOI-872b's transits will gradually disappear.

TTVs were originally proposed as a nontransiting planet detection method (2–4), but they have recently found more use in validating the transiting planet candidates from Kepler (24, 25). Kepler has previously inferred the presence of a nontransiting planet via TTVs, but showed that the measurements were unable to support a unique solution (26). Here we have demonstrated the full potential of TTVs as a method to detect nontransiting planets and precisely characterize their properties.

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19. We also tested the possibility of planets in the 1:1 mean motion resonance. We used an N -body integrator in this case, because perturbation theory described in (18) is not convergent for crossing orbits.
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21. To demonstrate this, we generated 10^6 realizations in the neighborhood of s2 and selected those consistent with the observed TTVs [as defined by $\chi^2 < \chi^2_{\text{min},s2} + \Delta\chi^2(99\%)$]. We found that the mass and orbit were well constrained from TTVs with $M_c \approx 1.8 \times 10^{-3} M_*$, $81.5 < P_c < 82.3$ days, $0.02 < e_c < 0.05$, and $7^\circ < i_c < 12^\circ$. We then calculated TDVs for the selected realizations and found that all were inconsistent with the measurements by $> 3\sigma$. In contrast, the realizations near s2 that were consistent with the observed TDVs had $i_c < 5^\circ$ and were inconsistent with the observed TTVs.
22. We attempted to fit the observed TTVs with a three-planet system. The obtained solutions recovered the two-planet case corresponding to s1 or s2, with the third planet being either too small or too distant to produce significant TTVs.
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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S12

Tables S1 to S5

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Tracking Cooper Pairs in a Cuprate Superconductor by Ultrafast Angle-Resolved Photoemission

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In high-temperature superconductivity, the process that leads to the formation of Cooper pairs, the fundamental charge carriers in any superconductor, remains mysterious. We used a femtosecond laser pump pulse to perturb superconducting $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+\delta}$ and studied subsequent dynamics using time- and angle-resolved photoemission and infrared reflectivity probes. Gap and quasiparticle population dynamics revealed marked dependencies on both excitation density and crystal momentum. Close to the d -wave nodes, the superconducting gap was sensitive to the pump intensity, and Cooper pairs recombined slowly. Far from the nodes, pumping affected the gap only weakly, and recombination processes were faster. These results demonstrate a new window into the dynamical processes that govern quasiparticle recombination and gap formation in cuprates.

The lifetime of Bogoliubov quasiparticles, the low-energy excitations of a superconductor, contains a wealth of information pertinent to the origin of superconductivity in a given material (*1*). This lifetime reflects two distinct processes: quasiparticle scattering and recombination. In the former, a quasiparticle scatters from one momentum state to another, conserving the fermionic particle number. Recombination, by contrast, refers to interactions in which two quasiparticles annihilate. To conserve energy and momentum, recombination must involve emission of other excitations—for example, phonons

or magnons—to which the quasiparticles are strongly coupled. Measurement of quasiparticle recombination rates as a function of their energy and momentum can, in principle, provide direct information about the interactions that induce Cooper pairing and superconductivity (*2*). Only recently, with the demonstration that angle-resolved photoemission spectroscopy (ARPES) can be performed with ultrashort laser pulse sources (*3–9*), have measurements with the necessary energy, momentum, and time resolution become possible. We present the results of experiments that use synchronized laser pulses to perform time-resolved

ARPES measurements of quasiparticle recombination and gap dynamics in the high-temperature superconductor $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+\delta}$.

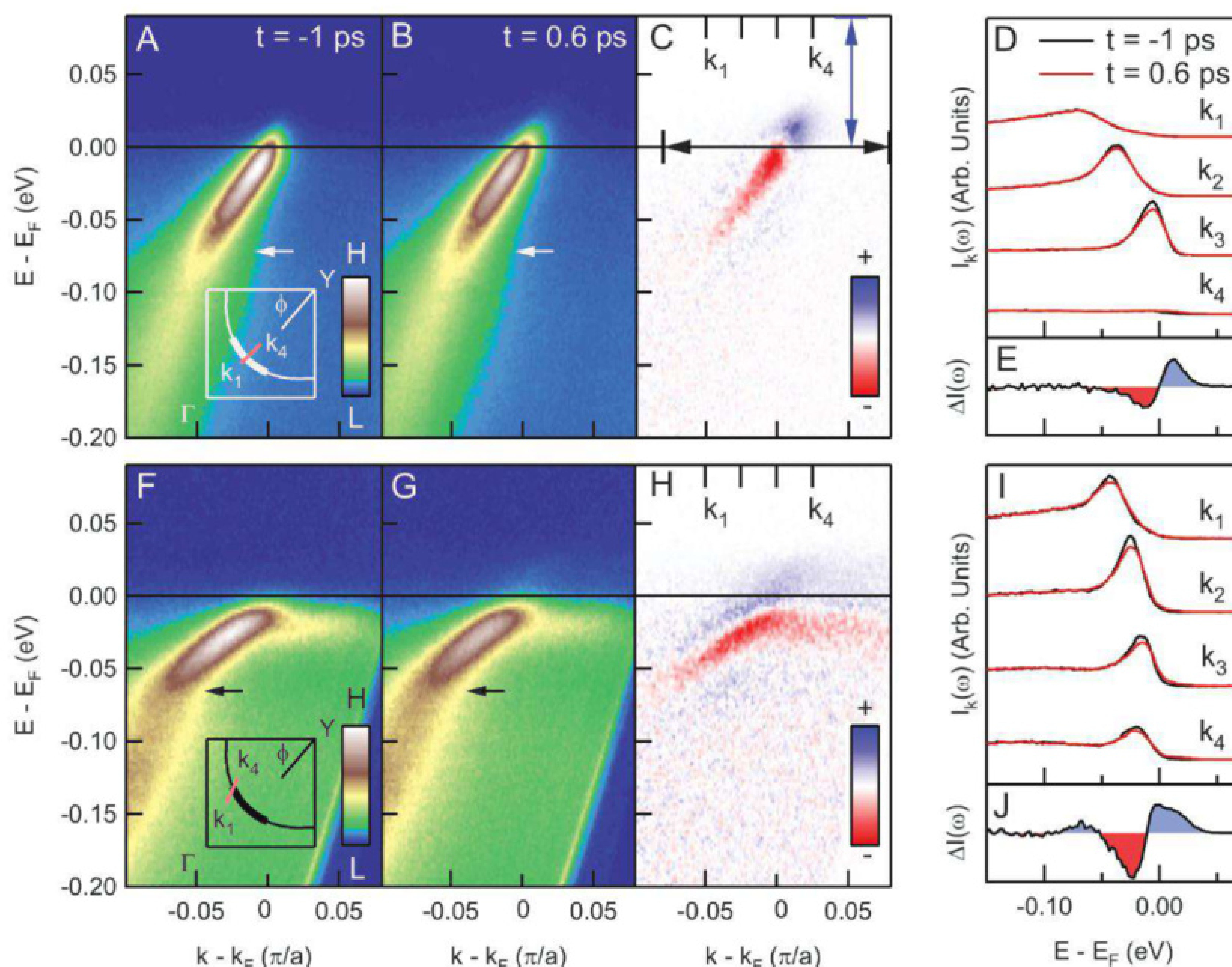
Measurements were performed at 18 K on an optimally doped sample with a critical temperature (T_c) of 91 K. A transient state is created with an infrared laser pump pulse ($\hbar\nu = 1.48$ eV, where $\hbar$ is Planck's constant and ν is the photon frequency), and measured via photoemission shortly thereafter, with a temporal resolution of 300 fs, using an ultraviolet probe pulse ($\hbar\nu = 5.9$ eV). The experiment benefits from high momentum and energy resolution ($0.003 \text{ \AA}^{-1}$ and 23 meV, respectively) and the ability to explore low pump fluences (2 to $15 \mu\text{J}/\text{cm}^2$).

Figure 1 shows typical equilibrium and transient ARPES dispersions ($t = -1$ and 0.6 ps, respectively) for cuts along nodal and off-nodal directions in k -space. Here, the time origin $t = 0$ coincides with the application of the pump pulse. The off-nodal cut has an equilibrium gap of 15 meV. In both cuts, a well-defined kink [marked by arrows in (A), (B), (F), and (G)] separates sharply defined coherent dispersive features

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Fig. 1. Typical ARPES dispersions before and after pumping for nodal ($\phi = 45^\circ$) and gapped ($\phi = 31^\circ$) regions of k -space. The incident pump fluence was $5 \mu\text{J}/\text{cm}^2$. (A) Equilibrium ($t = -1$ ps) and (B) transient ($t = 0.6$ ps) energy-momentum maps for the nodal state. Data are shown with identical color scales. The inset shows the location of the cut. The arrow marks the position of the dispersion kink. (C) Subtraction between (A) and (B). Blue indicates intensity gain and red intensity loss. (D) Energy distribution curves (EDCs) from k_1 to k_4 for equilibrium (in black) and transient (in red) states. EDCs are shifted vertically for ease of comparison. (E) Difference between transient and equilibrium EDCs, integrated across the double black arrow in (C). (F to J) Same as (A to E) but for a gapped (off-nodal) momentum cut. Spectra have been corrected for detector nonlinearity. The diagonal line in the lower right portion of (F) and (G) is the edge of the detector.



from poorly defined incoherent features, as is also visible in the selected energy distribution curves (EDCs) shown in (D) and (I). The following changes are evident in the transient spectra: (i) a decrease in intensity below the Fermi level (E_F) and slight broadening in the coherent spectra [(C to E) and (H to J)], similar to a previous report for nodal quasiparticles (5) and mainly confined below the kink binding energies (10, 11); (ii) an overall transfer of spectral weight across E_F [(C), (E), (H), and (J)], indicating the creation of transient quasiparticles; and (iii) a small shift of the spectral peak toward E_F in the off-nodal cut [(H and (I)], indicating a partial closure of the superconducting gap.

Figure 2 shows the temporal evolution of the superconducting gap in response to photoexcitation, as extracted from symmetrized EDCs at k_F , the Fermi wave vector (12). Panels (A and B) and (C and D) correspond to two representative cuts at $\phi = 32^\circ$ and $\phi = 27^\circ$, respectively, with ϕ defined according to the inset of (E). These spectra indicate very different responses of the gap amplitude to photoexcitation for the two cuts. The gap is relatively insensitive to fluences below $5 \mu\text{J}/\text{cm}^2$, but a fluence of $13 \mu\text{J}/\text{cm}^2$ induces a clear reduction in size. As shown in (E), the gap closer to the node decreases by 55% of its equilibrium magnitude, whereas the gap at $\phi = 27^\circ$ decreases by only 20%. This may indicate different dynamics inside and outside the Fermi arc, which is reported to end rather abruptly at $\phi = 30^\circ$ for samples of this doping (13, 14), although studies farther from the node are needed. Gap recovery rates are illustrated in Fig. 2F, where the curves from (E) have been inverted and rescaled by their maximum change. The initial recovery rate is slower for states closer to the node ($0.9 \pm 0.6 \text{ ps}^{-1}$) than for states farther from the node

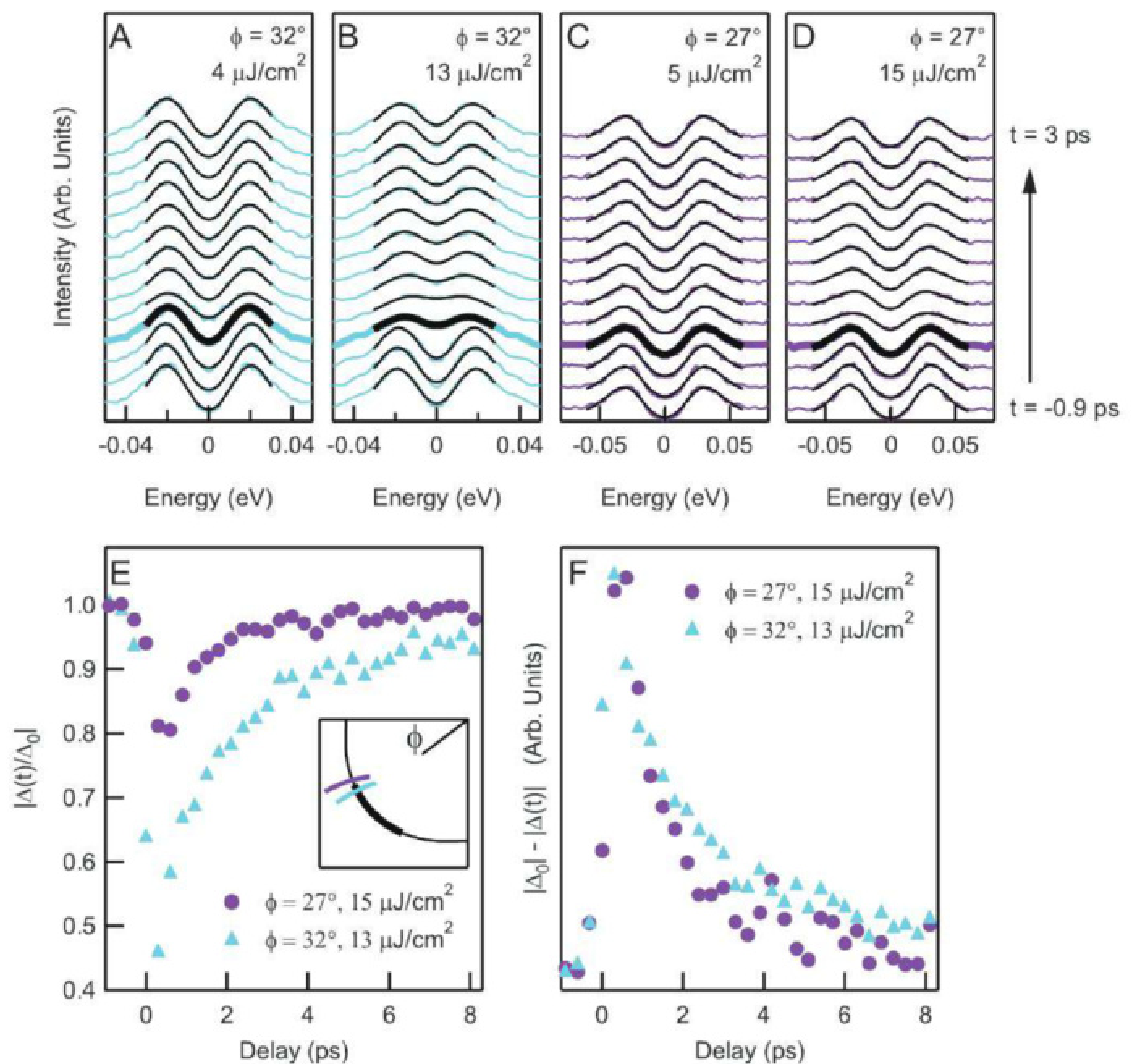
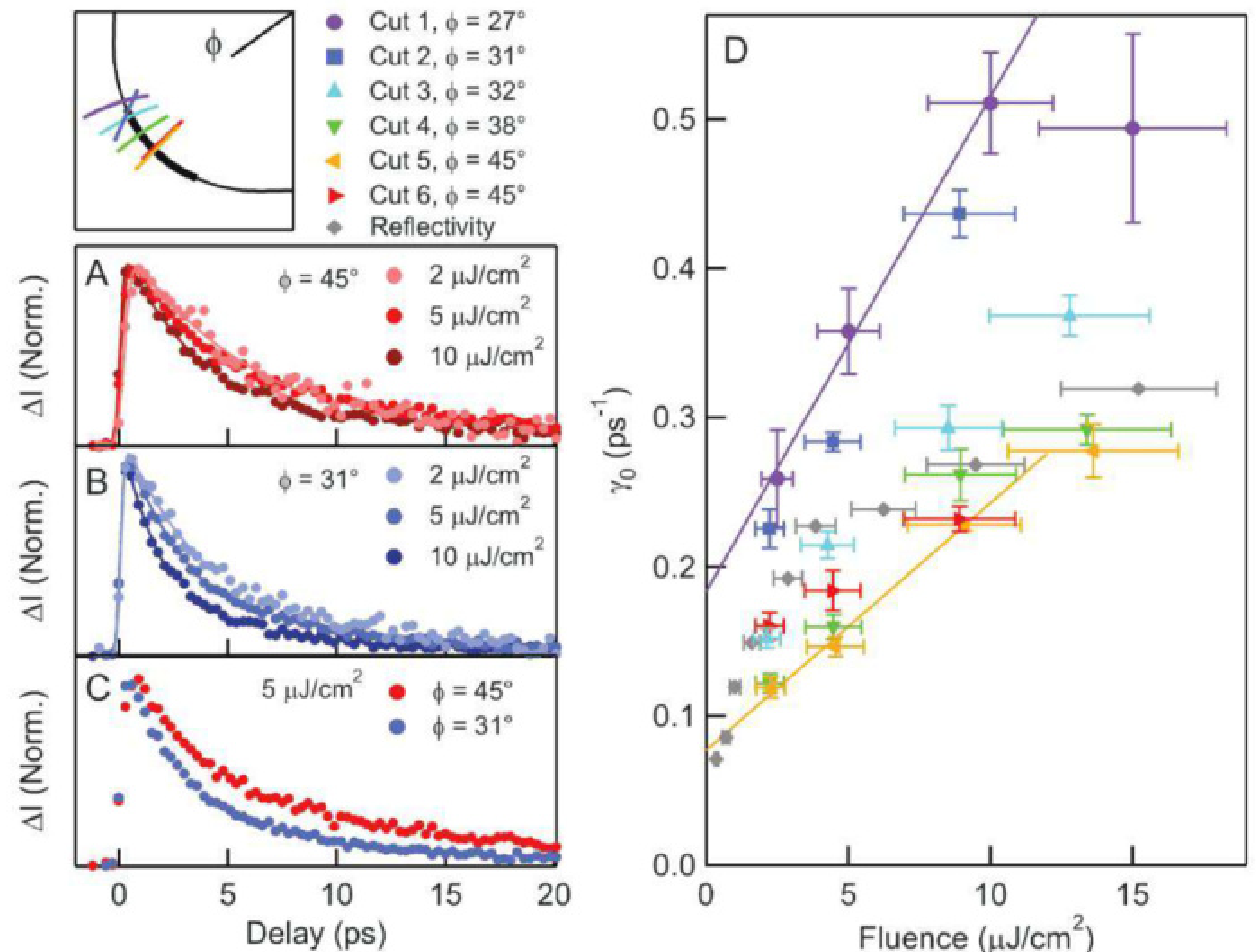


Fig. 2. Evolution of the superconducting gap after pump excitation. Symmetrized EDCs at k_F for $\phi = 32^\circ$ at low (A) and higher (B) fluence. The gap is obtained by fitting to a phenomenological model (12), but can be approximated by halving the distance between positive and negative peaks. Bold curves correspond to $t = 0$. For additional gap fitting details, see supplementary materials (15). (C and D) Analogous EDCs for a cut at $\phi = 27^\circ$. (E) Gap magnitude normalized by its equilibrium value versus pump-probe delay for momentum cuts at $\phi = 27^\circ$ and $\phi = 32^\circ$. (F) Gap magnitude, inverted and normalized by maximal change upon pumping in order to compare recovery rates.

Fig. 3. Quasiparticle recombination dynamics versus pump fluence and crystal momentum. ARPES data correspond to intensity change above E_F (ΔI) as integrated between the blue and black double arrows in Fig. 1C. Time-resolved reflectivity rates correspond to fractional change in reflectivity. (A) Nodal decay curves at 2, 5, and $10 \mu\text{J}/\text{cm}^2$, normalized to the same amplitude. (B) Analogous off-nodal decay curves ($\phi = 31^\circ$). (C) Overlay of nodal and off-nodal curves at the same fluence. (D) Initial decay rate γ_0 versus fluence, obtained by fitting decay curves at short times [for $\Delta I(t) \geq \Delta I_0/2$] to the convolution of a Gaussian and the function $f(t) = \Delta I_0 e^{-\gamma_0(t-t_0)} \Theta(t-t_0)$, where ΔI_0 and t_0 are additional fit parameters. Time-resolved reflectivity rates were multiplied by 3/2 in order to take the finite penetration depth of the optical probe into account (15).



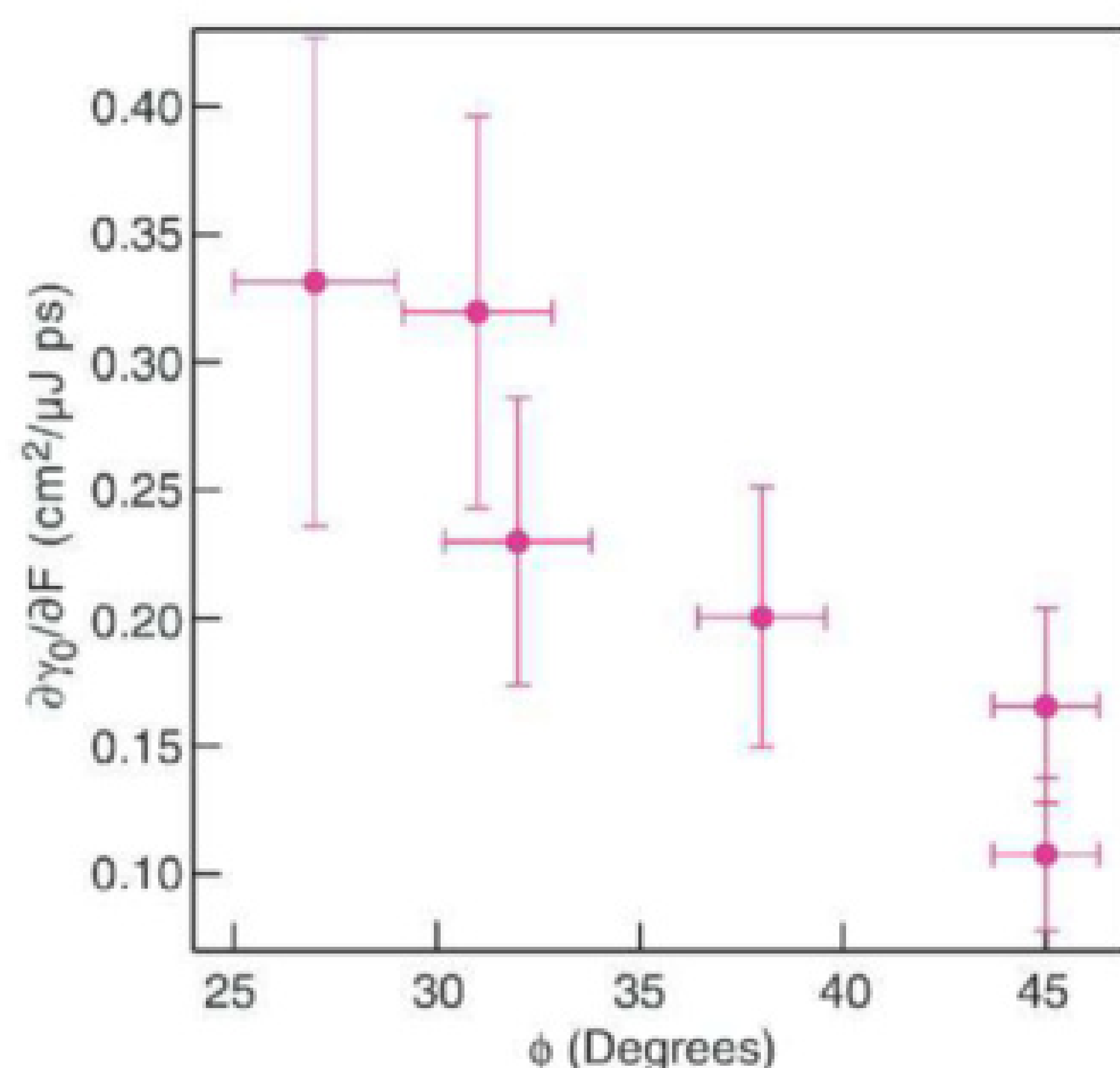


Fig. 4. Initial rate of increase $\partial\gamma_0/\partial F$ as extracted from straight line fits to the data in Fig. 3D for fluence $F < 12 \mu\text{J}/\text{cm}^2$. The horizontal axis corresponds to the Fermi surface angle.

($1.3 \pm 0.6 \text{ ps}^{-1}$), although the contrast is less apparent than that between amplitudes.

Figures 3 and 4 show quasiparticle recombination dynamics. In this low-fluence regime, the gap is almost unchanged for most of the recovery process, so quasiparticle recombination is largely decoupled from gap dynamics. Figure 3, A to C, shows the temporal evolution of the above- E_F spectral change ΔI for representative nodal and off-nodal k -space cuts, where ΔI is defined by the integrated intensity change across the blue and black double arrows in Fig. 1C. The spectral change is nearly symmetric above and below E_F in this fluence regime, so we focus on the intensity above E_F because of its superior statistics and smaller background. Faster decay rates occur at higher fluences and off-nodal momenta, an effect that cannot be explained by equilibrium heating (15).

Figure 3D summarizes the dependence of quasiparticle recombination on fluence and momentum. The rate γ_0 is defined by fitting the decay curves at short times to the convolution of a Gaussian and decaying exponential (16, 17). In line with Fig. 3, A to C, two prominent decay rate trends are apparent: (i) fluence dependence, with faster initial decay rates γ_0 occurring at higher fluences; and (ii) momentum dependence, with off-nodal decay rates increasing faster with fluence than those at the node. The first trend implies that intrinsic quasiparticle recombination processes are observed (17, 18). The second trend indicates that this recombination occurs more rapidly in off-nodal regions of k -space than at the node. The fluence dependence is also complementary to ultrafast studies using all-optical techniques, which report a marked dependence of decay rate on fluence, particularly in the low-fluence regime (16–20), and there is overall agreement between the ARPES results and a time-resolved reflectivity measurement taken on the same sample (gray circles in Fig. 3D). The decay rate measured by reflectivity is uniformly faster than nodal ARPES decay rates (cuts 5 and 6), but slower than the off-nodal rates (cuts 1 to

3), suggesting that optical spectroscopy provides an effective momentum-integrated average of the quasiparticle population. Notably, along both $\phi = 27^\circ$ and 32° directions, quasiparticle decay rates are slower than the gap recovery rates in Fig. 2: Compare $1.3 \pm 0.4 \text{ ps}^{-1}$ and $0.9 \pm 0.6 \text{ ps}^{-1}$ for the gap recovery versus $0.49 \pm 0.06 \text{ ps}^{-1}$ and $0.37 \pm 0.01 \text{ ps}^{-1}$ for the intensity recovery. This indicates that the superconducting gap recovers well before the nonequilibrium quasiparticle population drops to zero. A potentially related effect occurs at equilibrium in the Bardeen, Cooper, and Schrieffer (BCS) model, where the gap becomes large for T only slightly below T_c . Finally, the fluence and momentum dependencies reported here contrast with the findings of another time-resolved ARPES work (6), in which the quasiparticle recombination rate was reported to be independent of both fluence and momentum. The discrepancy might be explained by the higher fluences used in the previous work, which likely result in a complete closure of the superconducting gap, or by the coarser momentum and energy resolution compared to the present study.

As noted above [see also supplementary materials (15)], the fluence dependence of γ_0 means that ARPES decay rates are connected to intrinsic quasiparticle recombination processes. Time-resolved optical measurements indicate that the total (momentum-integrated) population of photoexcited quasiparticles $n_{\text{ex}}(t)$ can be described by a bimolecular rate equation (17, 18),

$$\frac{\dot{n}_{\text{ex}}}{n_{\text{ex}}} = -R(n_{\text{ex}} + 2n_T) \quad (1)$$

where R is a quasiparticle recombination constant, and n_T is the population of thermal quasiparticles. This is a special case of the Rothwarf-Taylor model of quasiparticle recombination (21), which has been successfully used to model dynamics of both conventional and high-temperature superconductors (17–20, 22–28). The general model also incorporates negative feedback from “hot” bosons ($\hbar\omega \gtrsim 2\Delta$) that are created by the quasiparticle decay process. For low fluence and temperature the model reduces to Eq. 1 under both weak feedback and strong feedback (boson bottleneck) scenarios, which produce differing interpretations of the coefficient R (17, 23). In both approximations, bimolecular recombination is the active ingredient in fluence-dependent dynamics.

In contrast to time-resolved optics, ARPES measures the momentum-dependent nonequilibrium quasiparticle density $n_k(t)$. The short-time fluence-dependent recombination dynamics are given by

$$\frac{\dot{n}_k}{n_k} \approx -\int R_{kk'} n_{k'} d^2k' \quad (2)$$

where $R_{kk'}$ is a modified recombination coefficient for the interaction between quasiparticles at specific points k and k' in reciprocal space. A weighted average of $R_{kk'}$ over k' is given by $\partial\gamma_0/\partial F$, the rate of increase of the initial decay rate γ_0 with fluence (15). Figure 4 shows an analysis of $\partial\gamma_0/\partial F$ as calculated by fitting straight lines to the data

in Fig. 3D for fluences $F < 12 \mu\text{J}/\text{cm}^2$. It is clear that $\partial\gamma_0/\partial F$ increases with decreasing Fermi surface angle ϕ , which means that the rate of recombination is enhanced as the quasiparticle momentum moves farther from the node.

One potential scenario for the momentum dependence of the recombination rates is that with increasing distance from the node, the quasiparticle energy and momentum approach resonance with charge or spin density wave fluctuations to which the electrons are strongly coupled. For example, a prominent neutron spin resonance is observed in $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+\delta}$ along the (1,1) momentum vector (29). Resonance between this mode and a quasiparticle pair would occur at a Fermi surface angle of about 12° , leading to the prediction of a peak in $\partial\gamma_0/\partial F$ at this Fermi surface angle. We believe that demonstrating that recombination can be mapped using time-resolved ARPES, and observing its strong momentum dependence, will further stimulate development of pulsed sources that are capable of reaching all the relevant regions of momentum space.

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Graphene Barristor, a Triode Device with a Gate-Controlled Schottky Barrier

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Despite several years of research into graphene electronics, sufficient on/off current ratio $I_{\text{on}}/I_{\text{off}}$ in graphene transistors with conventional device structures has been impossible to obtain. We report on a three-terminal active device, a graphene variable-barrier “barristor” (GB), in which the key is an atomically sharp interface between graphene and hydrogenated silicon. Large modulation on the device current (on/off ratio of 10^5) is achieved by adjusting the gate voltage to control the graphene-silicon Schottky barrier. The absence of Fermi-level pinning at the interface allows the barrier’s height to be tuned to 0.2 electron volt by adjusting graphene’s work function, which results in large shifts of diode threshold voltages. Fabricating GBs on respective 150-mm wafers and combining complementary p- and n-type GBs, we demonstrate inverter and half-adder logic circuits.

The triode, composed of a diode and a grid in a vacuum tube, was the first three-terminal active device that had been used to amplify and to switch electric signals, which led to the technical innovations for modern electronics in the early 20th century (1). Solid-state transistors and integrated circuits (ICs) based on silicon were more practical for complicated logic circuits and thus replaced triodes. Although silicon transistors have continued to improve their speed and integration density, they now near the potential limit where further reduction of channel length causes inevitable leakage currents (2). To present an alternative route for overcoming these challenges, we introduce a class of three-terminal devices based on a graphene-silicon hybrid device that mimics a triode operation. The key device function takes place at the electrostatically gated graphene/silicon interface where a tunable Schottky barrier controls charge transport across a vertically stacked structure. We named this barrier variable device, which is a solid-state descendant of the triode, “barristor.”

Graphene is a zero-gap semiconductor whose Fermi energy can be adjusted by electrostatic gating owing to its two-dimensional (2D) nature (3–5). Because graphene is metallic at a sufficiently large Fermi energy, a Schottky barrier (SB) forms at the interface between the doped graphene and the semiconductor (6–10). However, SB between graphene and a well-controlled semiconductor surface, such as hydrogen-terminated Si, is different from a conventional metal-semiconductor SB in two important ways. First, the formation of interface states is suppressed in graphene-semiconductor junctions (11) because the inter-

action between chemically inert graphene and a completely saturated semiconductor surface—that is, one without dangling bonds—is negligible (12). Second, graphene’s work function (WF) can be adjusted electrostatically over a wide range by tuning the Fermi energy (E_F) via the electrostatic field effect (13, 14). We could realize a graphene barristor (GB) by combining these two effects, as also shown in (15).

A schematic diagram of the GB structure and its top view are shown in Fig. 1, A and B. Single-layer graphene in contact with the source electrode forms the SB at its interface with the silicon surface at the drain contact. Two kinds of GBs can be formed depending on the silicon doping type: n-type GB at n-type silicon and p-type GB at p-type silicon. We used transmission electron microscopy (TEM) to develop an optimal transfer process for graphene onto Si substrates to create atomically sharp interfaces (inset of Fig. 1C), which minimizes atomic defects or silicon dioxide formation that can create charge trapping sites. Figure 1C displays a typical Schottky diode characteristic of a p-type GB with an optimized graphene/Si interface. The forward characteristic at a low bias showed a diode ideality factor $\eta_{\text{id}} \approx 1.1$ for this particular device. The ideality factor we obtained in our GB is considerably better than those reported in exfoliated graphene-Si junctions (9), confirming the high interfacial quality in our GBs.

The recent availability of large-scale graphene growth through chemical vapor deposition (CVD) techniques (16–20) allowed us to integrate graphene devices at wafer scales using conventional semiconductor microfabrication processes (21). Large-scale integration has not been possible for similar devices such as p-n diodes demonstrated in highly customized and suspended semiconducting

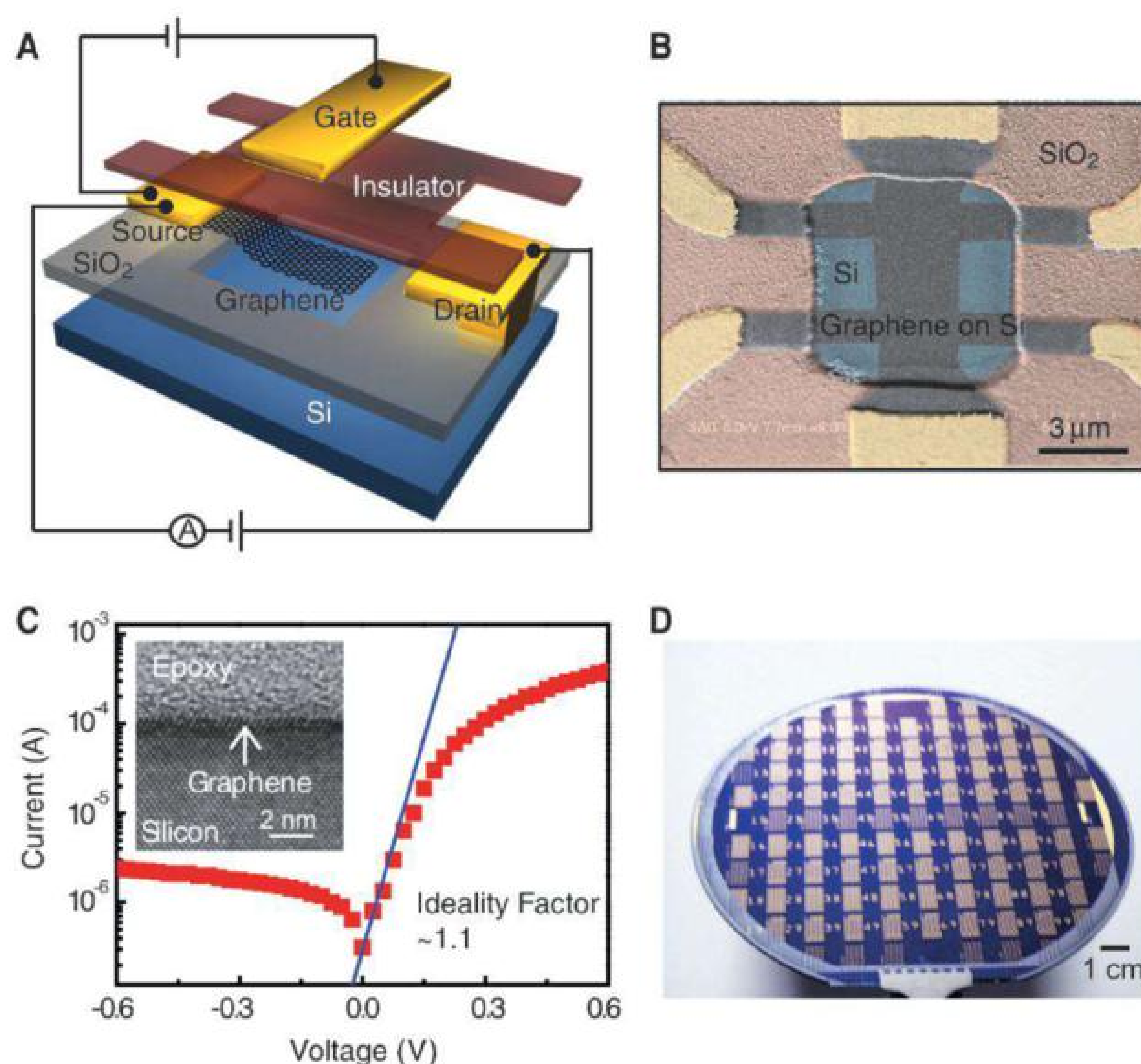


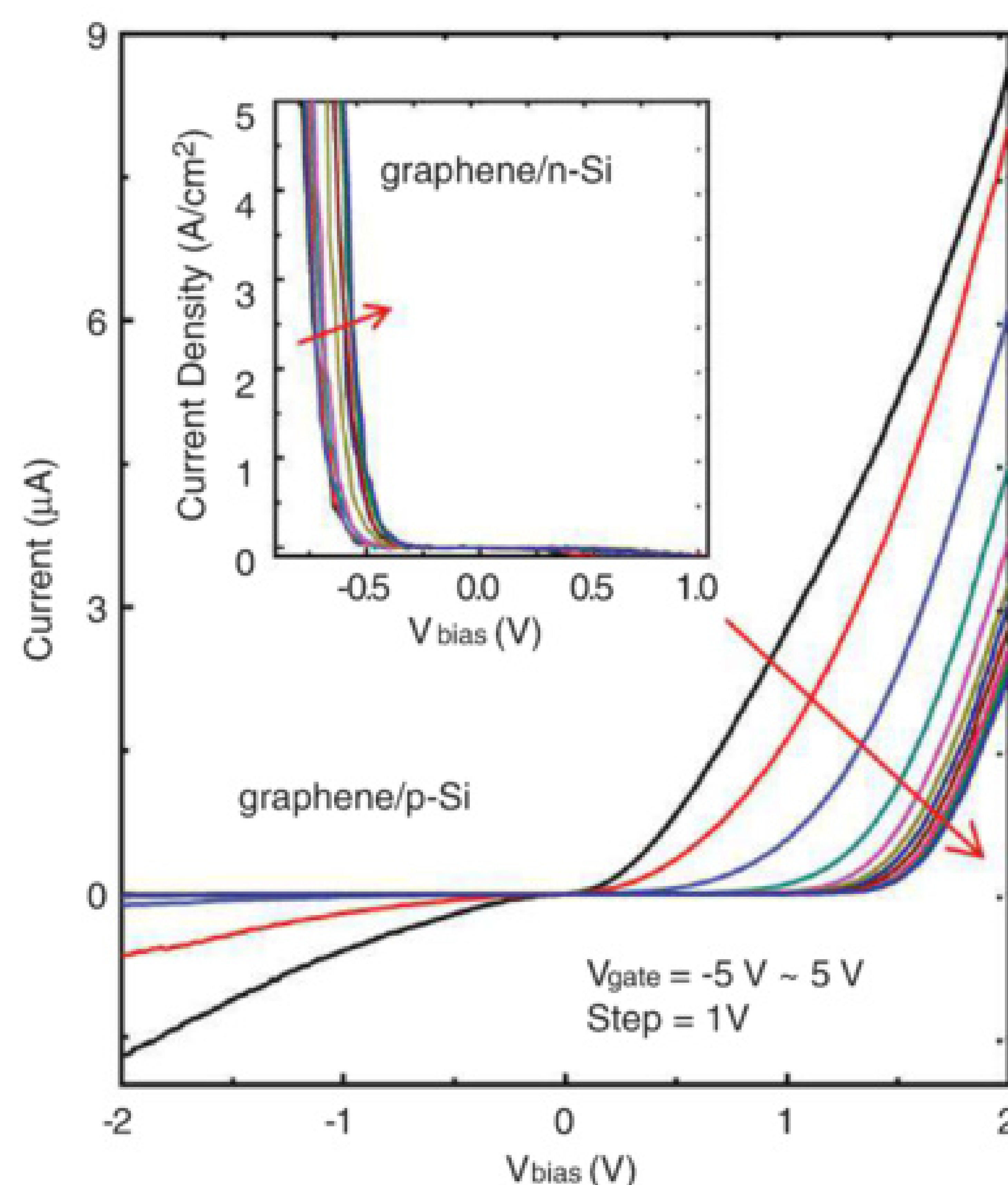
Fig. 1. Graphene barristor. (A) A schematic diagram to show the concept of a GB. (B) False-colored scanning electron microscopy image of the GB before the top gate fabrication process. (C) Current versus bias voltage characteristic of a GB at a fixed gate voltage $V_{\text{gate}} = 0$ V, showing a Schottky diode characteristic. The inset shows a TEM image of graphene/silicon junction. No native oxide or defect is seen in the image. (D) A photograph of ~2000 GB arrays implemented on a 6-inch wafer.

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Fig. 2. Graphene barristor characteristics. The current and bias voltage characteristics of p-type (main panel) and n-type (inset) GBs at various fixed V_{gate} values. V_{gate} varies in the range -5 to 5 V, with a step size of 1 V for each curve. The red arrow indicates the direction of increasing V_{gate} .



nanotubes (22, 23). We demonstrated integration of gate-tunable GB arrays on a 6-inch wafer by transferring a single-layer graphene grown by CVD (20, 24) onto a prepatterned Si substrate. Figure 1D shows an array of 2000 devices on a 6-inch (150-mm) wafer. At room temperature, the range of the ideality factor and the current density of GBs are found to be 1.1 to 4.0 and 10^1 to 10^4 A/cm², respectively (see figs. S3 to S5 in the supplementary materials) (25), where the variation is due to the different wafer batches. These GB devices exhibited $>90\%$ device yield, with excellent uniformity in terms of device characteristics across the wafer with appropriate current level (fig. S2).

We could electrostatically modulate the graphene's WF through the top gate electrode and gate dielectric above the graphene (14), which resulted in a variation on the SB height. Because the injection of the majority carriers from graphene to silicon is determined by the SB height ϕ_b , the top gate then directly controls the magnitude of the current across the source and the drain. The main panel of Fig. 2 shows the characteristic of p-type GB at various gate voltages (V_{gate}). The rectification behavior of the GB was demonstrated in that the GB current (I_{SD}) increased steeply as the bias voltage (V_{bias}) surpassed the "turn-on" voltage, V_{TO} . V_{TO} could be adjusted between 0 V and 1.3 V as V_{gate} was modulated between -5 and 5 V, the range needed to keep this GB in p-type operation. Similar triode behavior was observed in n-type GBs (Fig. 2 inset), where the bias polarity was reversed because electrons become the majority carrier. The large modulation of the GB current was indicative of a large variation in the SB height, caused by the electric field effect (EFE) from V_{gate} .

To quantitatively analyze the device characteristic, we used the diode equation

$$I = AA^* T^2 \exp\left(\frac{-q\phi_b}{k_B T}\right) \left[\exp\left(\frac{qV_{\text{bias}}}{\eta_{\text{id}} k_B T}\right) - 1 \right]$$

where A is the area of the Schottky junction, A^* is the effective Richardson constant, q is the elementary charge, k_B is the Boltzmann constant, and T is the temperature. Quantitative analysis of the SB height ϕ_b can be done by investigating the temperature dependence of the GB current in the reverse bias saturation regime [$\exp(qV_{\text{bias}}/\eta_{\text{id}} k_B T) \ll 1$]. Here, the diode current becomes insensitive to V_{bias} and $I_{\text{sat}} \propto T^2 \exp\left(\frac{-q\phi_b}{k_B T}\right)$. Figure 3A shows a plot of $\ln(I_{\text{sat}}/T^2)$ versus $q/k_B T$ in the reverse bias saturation regime. We estimated the SB height ϕ_b for a given gate voltage V_{gate} from the slope of each curve. Figure 3B shows the resulting SB heights obtained as a function of V_{gate} . V_{gate} increased from 0 to 5 V, and ϕ_b decreased substantially from 0.45 eV to 0.25 eV. We attributed the drastic change in ϕ_b to the EFE-induced Fermi level change, ΔE_F . Indeed, as shown in Fig. 3B, the measured variation of ϕ_b , $\Delta\phi_B =$

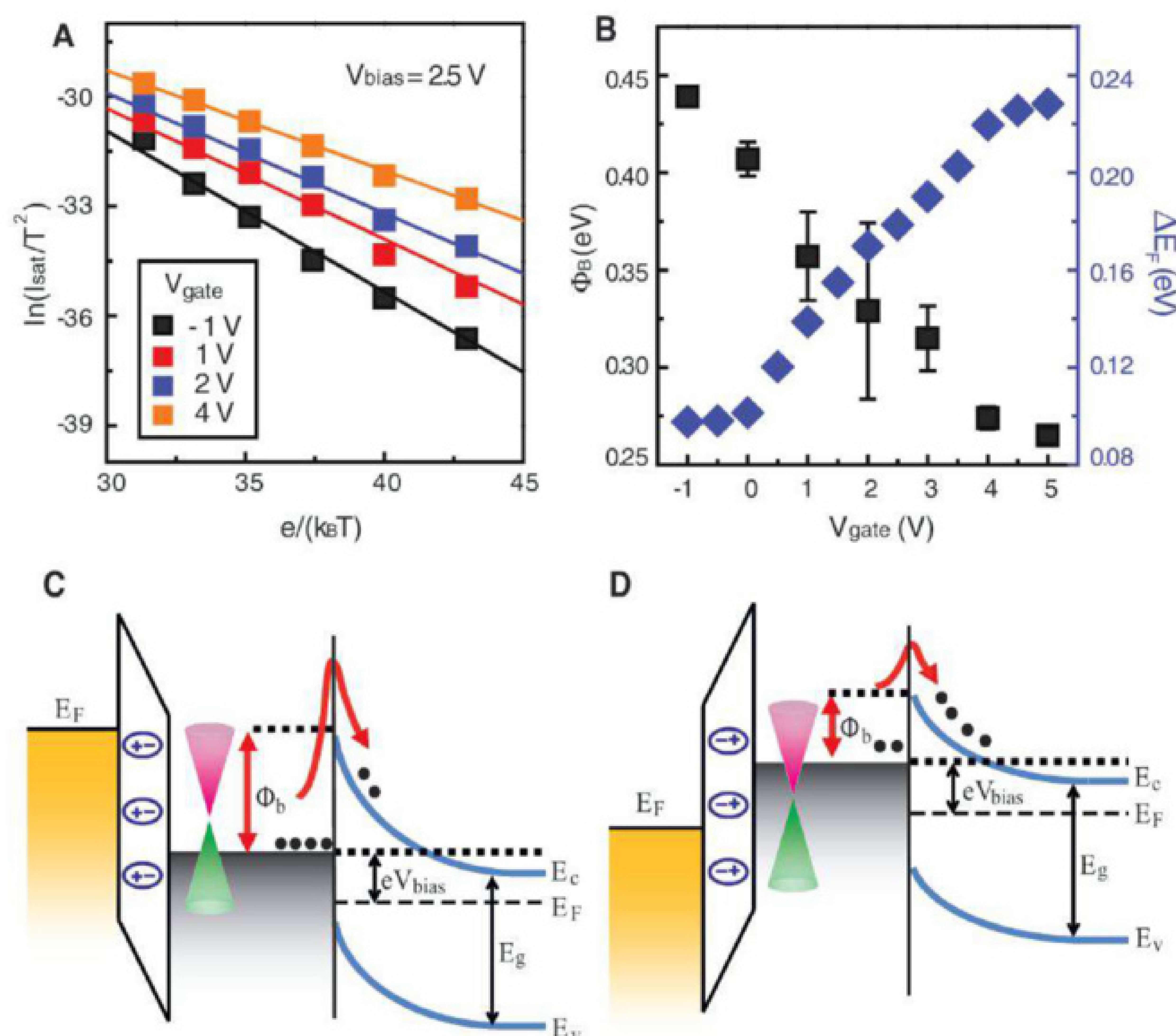
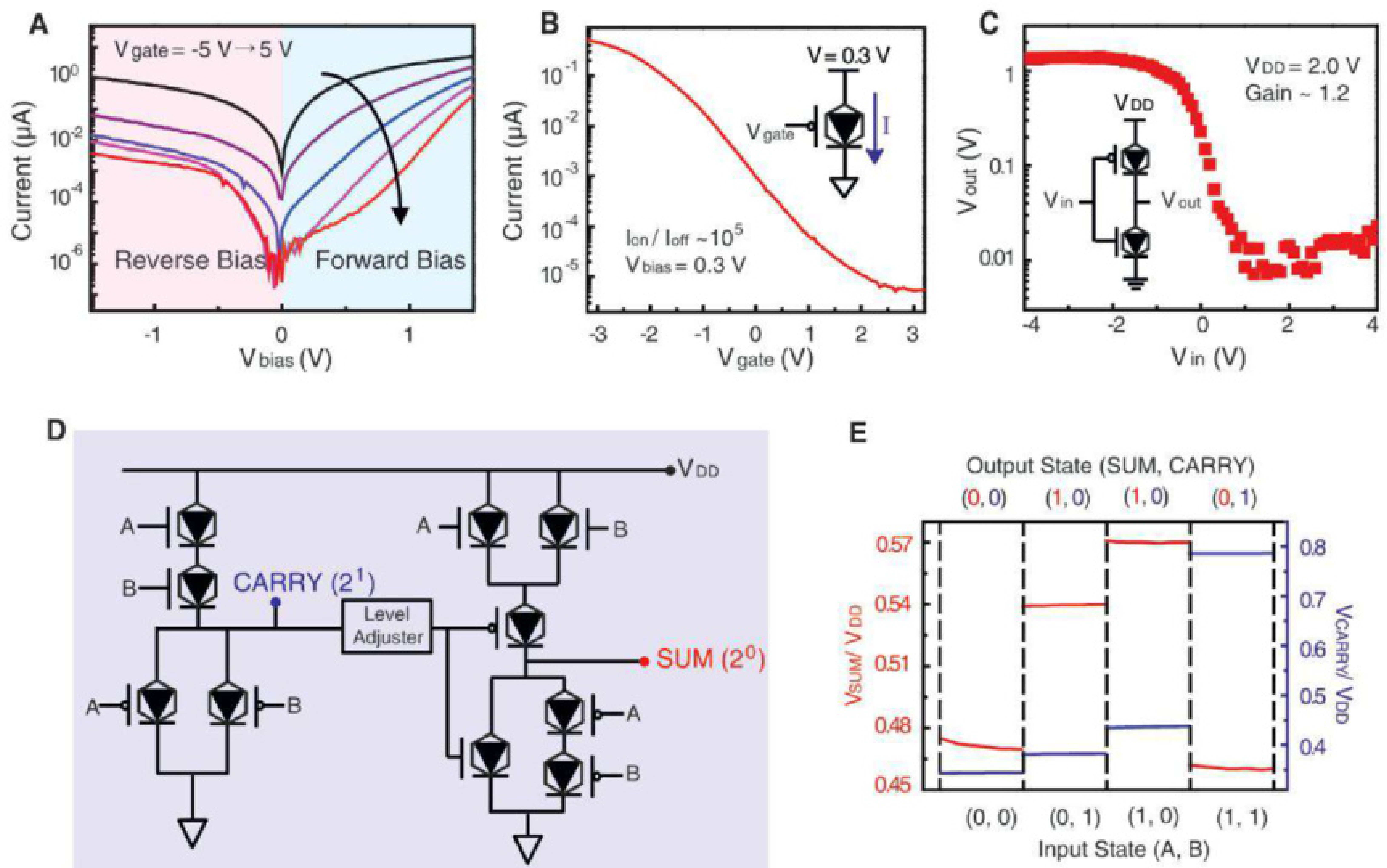


Fig. 3. (A) Temperature-dependent diode characteristic. The saturation current of the n-type GB, I_{sat} , is obtained by measuring the current at $V_{\text{bias}} = 2.5$ V at various temperature and gate voltage values. Different colors are used for different V_{gate} from -1 to 4 V with 1 - to 2 -V step variation. The line fit for each V_{gate} value is drawn to yield the Schottky barrier height from the slope of the fitted line. (B) The SB height obtained from the fit in (A) as a function of V_{gate} (black squares, left y axis). The graphene Fermi energy on the right y axis is obtained from Hall measurement at the same gate voltage V_{gate} . Monotonic increase and decrease of observed SB height is consistent with the band diagrams in (C) and (D), respectively. (C and D) Schematic band diagrams of GB with the EFE generated by the gate on the top of graphene. Applying negative voltage on the gate induces holes in graphene, increasing its work function and increasing the Schottky barrier height. As a result, the reverse current across the Schottky barrier decreases (C). Positive gate voltage decreases the Schottky barrier height and increases reversed current (D).

Fig. 4. (A) Switching behavior of p-type GB in reverse (orange background) and forward (blue background) bias regimes. The GB current is plotted against the source drain bias at various fixed gate voltages. V_{gate} varies in the range of -5 to 5 V, with a step of 2 V. The black arrow indicates the direction of increasing V_{gate} . (B) The forward diode current as a function of gate V_{gate} at fixed bias $V_{\text{bias}} = 0.3$ V. Unipolar control of forward current with the ratio of 10^5 is obtained. (C) Inverter characteristics obtained from integrated n- and p-type GBs and schematic circuit diagram for the inverter. Positive supply voltage (V_{DD}) is connected to p-type GB, and the gain of the inverter is ~ 1.2 . (D) Schematic of circuit design of a half-adder implemented with n- and p-type GBs. (E) Output voltage levels for SUM and CARRY for four typical input states.



$\phi_b - \phi_b(\Delta E_F = 0)$, was well correlated to the change of ΔE_F , obtained independently by converting the measured Hall carrier density (n_H) using $\Delta E_F = \hbar v_F \sqrt{\pi n_H}$, where $v_F = 10^6$ m/sec was used for the Fermi velocity of graphene. We observed that $\Delta \Phi_B \approx -\Delta E_F$ for a wide range of V_{gate} (fig. S7), which suggests that the WF modulation of graphene in the absence of Fermi-level pinning is fully responsible for the variation of ϕ_b as depicted in Fig. 3, C and D.

The absence of Fermi-level pinning, one of the major sources for high device resistance in silicon electronics (26), comes from the suppression of the surface states at the interface of silicon and graphene. In our GB, we could eliminate the “Fermi-level pinning” and manipulate the SB height as in the ideal Schottky-Mott limit (Fig. 3B), where the SB height is controlled through the selection of metal and semiconductor with appropriate WFs (27, 28).

Two different types of GB operations are possible, as shown in Fig. 4A. The first type uses the GB in the reverse-biased regime (orange shaded region in Fig. 4A). Here, a conventional FET-like device operation could be possible; that is, the GB current, I , had the tendency to saturate at large reverse bias voltages, and the on-state current, I_{on} , had been modulated by V_{gate} . The off-state current, I_{off} , was determined at the greatest attainable SB, which yielded an $I_{\text{on}}/I_{\text{off}}$ ratio of ~ 300 in Fig. 4A. The second type of operation could be realized by using the device in the forward biasing region (the blue shaded region in Fig. 4A). In this regime, the diode current did not saturate as V_{bias} increased but deviated from a typical FET-like device opera-

tion. However, near the diode turn-on regime, I varied by several orders of magnitude as V_{gate} changed, resulting in a switching operation with a large $I_{\text{on}}/I_{\text{off}}$ ratio. Figure 4B shows the switching characteristic of the diode current in a forward-biased p-type GB, demonstrating a high $I_{\text{on}}/I_{\text{off}}$ ratio of $\sim 10^5$. We note that the large $I_{\text{on}}/I_{\text{off}}$ ratio and small off-state current overcame the key obstacle in graphene-based electronics.

Three-terminal operation of GB offers various integrated device functionalities. Similar to the complementary metal-oxide semiconductor inverter operation, a series connection of n- and p-type complementary GBs inverted the input signal V_{in} to its inverse V_{out} (Fig. 4C). The low V_{out} originated from the high on/off current ratio of GBs, which implies that only a small amount of static off-state power would be consumed. As for more complicated logic applications, we demonstrated a half-adder circuit built from n- and p-type 10 GBs. The schematic with two inputs (A and B) and two outputs (SUM and CARRY) is shown in Fig. 4D. The measured output voltage levels are represented in Fig. 4E. These results suggest that the GB can provide a route to realize high-speed logic applications (29, 30) based on graphene-semiconductor hybrid devices.

In conclusion, our GB suggests the possibility of a graphene logic device by achieving a high on/off ratio of $\sim 10^5$, which exceeds the minimum requirement for logic transistors. Furthermore, the on/off ratio and the current density can be improved with well-developed semiconductor processes because there is not a fundamental (or structural) limit. GB also has an advantage

in lateral scaling because the barrier is formed vertically.

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Supplementary Materials

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Supplementary Text
 Figs. S1 to S10
 References

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Tailoring Electrical Transport Across Grain Boundaries in Polycrystalline Graphene

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Graphene produced by chemical vapor deposition (CVD) is polycrystalline, and scattering of charge carriers at grain boundaries (GBs) could degrade its performance relative to exfoliated, single-crystal graphene. However, the electrical properties of GBs have so far been addressed indirectly without simultaneous knowledge of their locations and structures. We present electrical measurements on individual GBs in CVD graphene first imaged by transmission electron microscopy. Unexpectedly, the electrical conductance improves by one order of magnitude for GBs with better interdomain connectivity. Our study suggests that polycrystalline graphene with good stitching may allow for uniformly high electrical performance rivaling that of exfoliated samples, which we demonstrate using optimized growth conditions and device geometry.

Most three-dimensional electronic materials produced in macroscopic quantities are not homogeneous but incorporate numerous classes of dislocations and defects that degrade electrical performance (1). Although large-scale graphene films produced by chemical vapor deposition (CVD) (2, 3) might be expected to be nearly defect free, recent transmission electron microscopy (TEM) studies (4, 5) have shown that these films are polycrystalline. Electrical transport between single-crystal domains could be affected by scattering at the grain boundary (GB), as has been shown theoretically (6–9). Although TEM has provided a fast and accurate means to identify and image the structure of GBs in CVD graphene, the electrical impact of GBs has so far been studied only indirectly in experiments. Previous work by Huang *et al.* detects no measurable electrical resistance from GBs within instrument limits (4), and ensemble measurements done by various groups find very weak correlation between the average domain size of the graphene film and overall device mobility (4, 10). In contrast, Yu *et al.* and Jauregui *et al.* inferred the presence of GBs from the shape of partially grown graphene islands and extracted a finite GB resistance from their measurements (11, 12). The ambiguity in these findings arose from a lack of knowledge of the precise domain morphology

for the graphene measured. To this end, we have devised an experimental scheme to first image (using TEM) and then electrically address individual domains and GBs in polycrystalline graphene. Such a capability is crucial, because graphene domain structures generated during synthesis form nontrivial patterns that are strongly dependent on growth conditions and difficult to predict a priori.

In Fig. 1A, we show false-color dark-field TEM (DF-TEM) images of graphene films grown under three different conditions [see supplementary materials (13)] taken in a manner similar to Huang *et al.* (4). Each colored region corresponds to a separate graphene crystalline domain with distinct lattice orientation. The image is generated by using an aperture in the back-focal plane of the microscope to collect electrons diffracted from only a narrow range of angles by the graphene lattice. In general, different graphene domains produce a diffraction pattern rotated with respect to one another, so each domain can be imaged separately, colored, and then combined.

In growth A, graphene was synthesized under high reactant flow rates, which produced fast growth and also small average domain size $D \approx 1 \mu\text{m}$. Graphene from growth B was synthesized in a diluted methane environment, whereas growth C was further enclosed in copper foil, after Li *et al.* (14), resulting in slower growths. The latter films were terminated after only partial surface coverage to highlight their growth structures. In subsequent microscopy and electrical measurements, however, we used continuous films, for which growth B yielded $D \approx 10 \mu\text{m}$ and growth C, $D \approx 50 \mu\text{m}$. The overall shapes of partially grown graphene islands in growth B were polygons, whereas growth C generally formed flowered islands (fig. S1). Despite

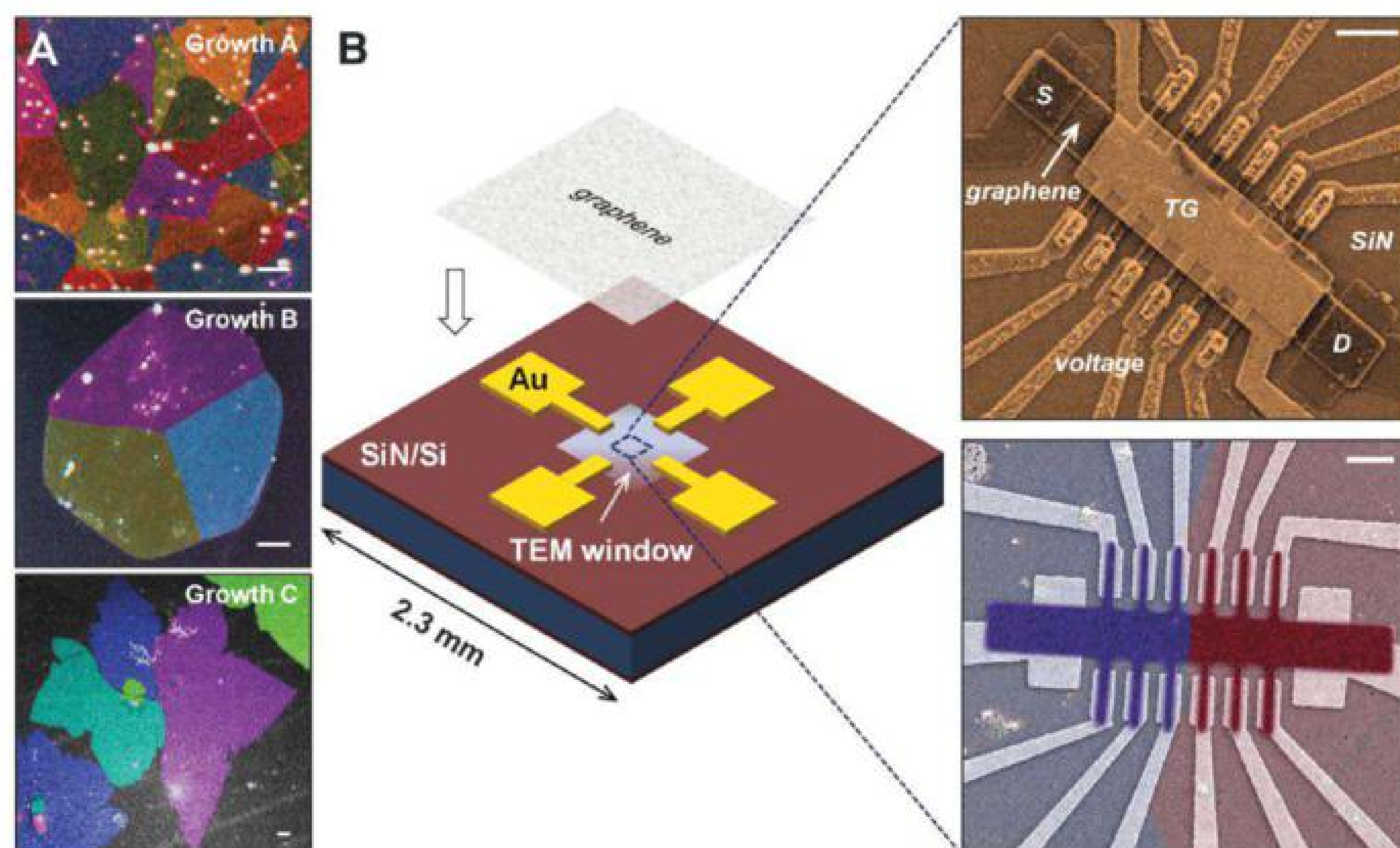


Fig. 1. (A) Composite false-color DF-TEM images of CVD graphene produced using three different growth conditions—A, B, and C—yielding average domain size D of 1, 10, and $50 \mu\text{m}$, respectively, in continuous films. (B) (Left) Schematic of specially fabricated TEM chip compatible with electron-beam lithography and electrical measurements. (Top right) SEM image of top-gated, graphene Hall bar device. (Bottom right) Overlaid SEM and DF-TEM images showing device crossing a single GB of two domains from growth C. Scale bars, $1 \mu\text{m}$.

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these differences, DF-TEM shows that even a single graphene island can contain several distinct domains, revealing the complexities of graphene growth inherent in even seemingly simple growth structures.

To study the impact of individual GBs on electronic properties, electrical measurements and TEM imaging need to be done in conjunction. However, complicated sample preparation processes and constraints arising from chip geometry make TEM studies currently incompatible with other experimental schemes, such as nanofabrication. Here, we describe a method to electrically address a material with nanometer resolution after TEM characterization. In Fig. 1B (left), we show a schematic of a specially fabricated TEM chip (see supplementary materials and fig. S2) that is 2.33 mm wide on each side, 200 μm thick, and fits standard TEM holders. In the center is a fully suspended, silicon nitride (SiN) TEM window (80 by 80 μm , 20 nm thickness) with metalized alignment marks. On the periphery are large electrical leads and contacts patterned by optical lithography. After graphene was transferred and imaged, we selected an area of interest and used electron-beam lithography to pattern a field-effect transistor device with four-terminal geometry. Raman spectra taken after our TEM imaging suggests that our graphene was not damaged during this process (fig. S3). In three separate lithography steps, we (i) defined electrodes, (ii) patterned the graphene, and (iii) defined a top gate. A completed structure is shown in the scanning electron

microscopy (SEM) image on the top right of Fig. 1B. By combining TEM and standard fabrication methods, we electrically addressed individual domains and GBs in CVD graphene with $\approx 50\text{-nm}$ accuracy. In the bottom right, we show an SEM image of a representative device (before defining the top gate) overlaid with a false-color DF-TEM image of the underlying graphene, consisting of a single GB between two large domains. The areas with faded colors represent graphene that was subsequently etched away, leaving behind only the high-contrast pattern in the center.

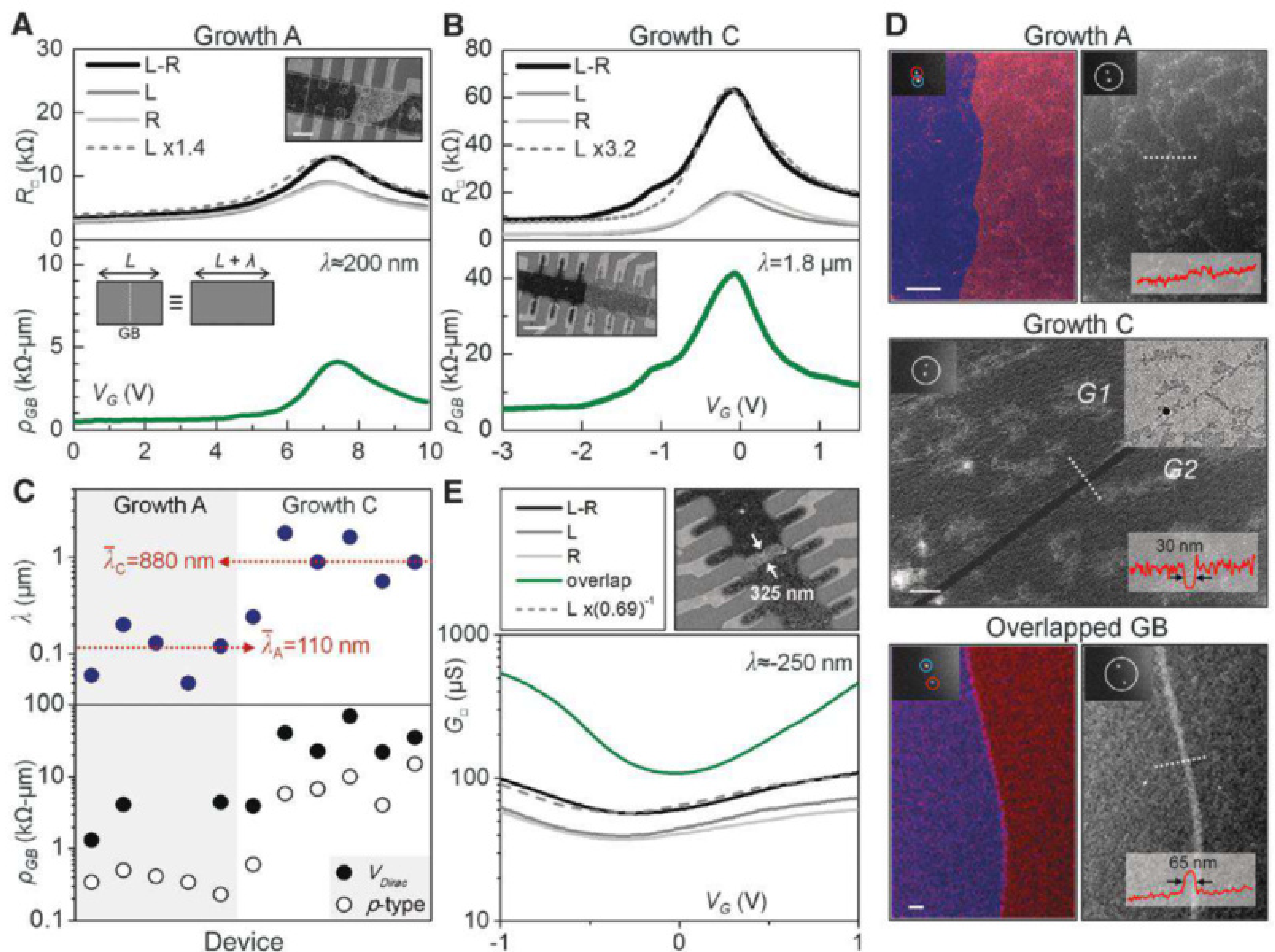
We first performed room-temperature transport measurements on a GB from growth A ($D \approx 1 \mu\text{m}$). In the upper inset of Fig. 2A, we show the composite image of a particular device consisting of an individual GB. In the top panel, we plot four-terminal sheet resistance $R_{\square}$ versus gate voltage for the left (L) and right (R) domains, as well as that across the GB (L-R), measured simultaneously for the same gate voltage sweep. The single-domain measurements showed field-effect behavior that was typical for graphene with a Dirac point at $V_{\text{Dirac}} \approx 7 \text{ V}$. More strikingly, the two showed nearly identical values for the entire gated range. The cross-domain measurement showed similar qualitative behavior with a comparable Dirac position. However, L-R exhibited an increased resistance, particularly at gate biases near V_{Dirac} , that we attributed to additional scattering caused by the GB. Also, L-R seemed to scale from single-domain resistivity ($\rho_{\square}$) by a constant factor of 1.4 for all gate values, as shown by

the dashed curve. Finally, by subtracting the averaged L and R values from L-R, we can extract the resistivity per micrometer length of the GB itself, ρ_{GB} , which we plot in the bottom panel as a function of gate voltage. ρ_{GB} exhibited a similar gate-tunable behavior as $\rho_{\square}$: It is 4 $\text{k}\Omega\text{-}\mu\text{m}$ at V_{Dirac} and decreased with doping, reaching a saturated value of 0.5 $\text{k}\Omega\text{-}\mu\text{m}$ in the p -type regime.

The results of our measurement can be interpreted to describe the presence of a GB as simply an extension of the conductance channel defined by an effective length $\lambda = \rho_{\text{GB}}/\rho_{\square}$. When a device of dimensions L and W crossed a GB, resistance increased from the intrinsic, monocrystalline resistance $R = \rho_{\square}(L/W)$ to $R' = \rho_{\square}(L/W) + \rho_{\text{GB}}/W = \rho_{\square}(L+\lambda)/W$. Hence, the channel length effectively increased by λ , and both the electrical conductance and the carrier mobility were reduced by a factor $R'/R = 1+\lambda/L$. Because R'/R closely followed a fixed scaling with gate voltage in our measurements, λ was approximately constant and independent of carrier density (fig. S4). This effect, as diagrammed in the lower inset of Fig. 2A, is a key finding of this report. In particular, the introduction and determination of λ (representing GB connectivity), along with average domain size D , will allow tailoring of overall electrical transport in devices of all length scales. For this particular device, we extracted a value of $\lambda \approx 200 \text{ nm}$.

Different electrical behavior was observed for analogous measurements at a GB from growth C ($D \approx 50 \mu\text{m}$), which we show in Fig. 2B. Here, L

Fig. 2. (A) (Top) Four-terminal sheet resistance $R_{\square}$ of device crossing a single GB from growth A (SEM/DF-TEM shown in inset; scale bar, 1 μm) measured in left (L), right (R), and across (L-R) domains as a function of gate bias. L-R scales L by a factor of 1.4. (Bottom) Extracted gate-dependent GB resistivity ρ_{GB} . GB acts to increase channel length by $\lambda \approx 200 \text{ nm}$ at all gate biases. (B) Corresponding measurement of more resistive GB from growth C device (scale bar, 1 μm). L-R scales L by 3.2; $\lambda = 1.8 \mu\text{m}$. (C) λ and ρ_{GB} (at V_{Dirac} and p -type doping) across 11 single-GB devices from growths A and C. Overall, GBs from growth C are an order of magnitude more resistive. (D) (Top and middle) Higher resolution DF-TEM images of GBs from suspended growth A and C samples show better stitching from growth A. (Bottom) DF-TEM of overlapped GB often observed in growth C. The diffraction spots and relative aperture positions used to form the images are shown as insets. Scale bars, 100 nm. (E) Electrical measurements of device consisting of GB overlapped by 325 nm show improved GB conductance. $\lambda \approx -250 \text{ nm}$.



and R also showed similar gate dependences, but L-R was considerably greater at all gate values, signifying increased scattering at the GB. In fact, we extracted a gate-dependent GB resistivity (5 to 40 k Ω - μ m) that was overall an order of magnitude greater than that measured for growth A. Nevertheless, $R'/R \approx 3.2$ was again roughly constant with gate bias, from which we determined $\lambda = 1.8$ μ m for this GB, or twice as long as the device channel itself.

We fabricated 11 graphene devices with a single GB and performed similar measurements (five devices from growth A and six from C). In Fig. 2C, we plot their GB resistivities measured both at V_{Dirac} and with p -type doping. Although we observed a range of different values, GBs from growth C were an order of magnitude more resistive overall. We also plot the corresponding gate independent λ values: The mean for growth A samples was 110 nm, and it was 880 nm for C. In contrast, we observe no strong correlation between ρ_{GB} and different growth conditions (fig. S5).

The poorer conductance of GBs from growth C can be explained by careful investigation of their GB structure. Although graphene in growth A exhibited much greater polycrystallinity, it yielded highly uniform coverage of the underlying copper surface. However, for growth C, we often observed many areas between islands with small gaps and overlaps, suggesting a re-

duced tendency for different domains to stitch (fig. S6). To study the morphology of individual GBs with higher resolution, we suspended separate graphene samples from the same growths on top of perforated SiN chips to examine with DF-TEM. We found distinct classes of GB behavior from growths A and C.

In Fig. 2D (top left), we show a DF-TEM image of a GB between two colored domains from growth A, with their respective diffraction spots circled in the inset. On the right, we take an image of the entire region by selecting both spots simultaneously and see a relatively featureless boundary region, which is further highlighted by the flat intensity profile taken across the boundary along the marked line. Recently, Huang *et al.* have imaged a particular GB of this type with angstrom resolution and demonstrated that it can form an atomically sharp junction locally at the nanometer scale (4). In contrast, domains from growth C showed a markedly different behavior, as seen in the middle panel. Here, two domains have physically connected, as can be seen in the bright-field image in the inset, and showed no slack at the boundary despite being suspended. However, selecting the diffraction spots for both domain orientations simultaneously revealed a dark strip 30 nm wide where the domains join. This result implies that this extended boundary region had a structure different from that of the crystals on either side, suggesting that the domains were joined together either by graphitic material at another orientation or by amorphous material, although atomic resolution imaging would be needed to confirm this directly. The presence of grain boundaries with greater crystalline discontinuity for growth C appeared to be a general trend (fig. S7). Additionally, we have observed growth C domains to connect via an overlapping region. An example is shown in the bottom panel of Fig. 2D. Again, the overlap was seen most clearly by selecting diffraction spots from both domains simultaneously, because the double-layered re-

gion appeared twice as intense in the dark-field image. Here, one domain extended 65 nm on top of the other, although overlaps as large as 1 μ m were observed for longer growths. Similar behavior has also been observed by Robertson *et al.* using atomically resolved TEM imaging (15). We therefore conclude that the electrical properties of GBs in CVD graphene are not universal but are, rather, keenly sensitive to growth conditions and reflect the quality of the connection between domains. In particular, the high-reactivity environment from growth A that yielded a faster growth rate could also contribute to better inter-domain stitching.

We have also fabricated a device across two domains from growth C that have overlapped at their boundary, which we show in Fig. 2E. Here, the domains overlapped by 325 nm. Instead of an increased cross-domain resistivity, we observed a conductance that was enhanced by a factor of 1.45, which implies an effective negative $\lambda \approx -250$ nm. We also plotted the square conductance from the overlapped graphene exclusively by removing contributions from the L and R domains, and we saw that it was an order of magnitude greater than single-layered graphene. This result suggests that the scattering properties in double-layered graphene are improved from single-layer, so reliable synthesis of grain boundaries with large overlap, if possible, would be an exciting advance. At the same time, we would also expect this effect to be sensitive to the length of overlap, because a narrow overlap could still potentially hinder interdomain transport.

To better understand the origin of GB resistance for our devices with nonoverlapping domains, we plotted in Fig. 3A the GB conductivity $\sigma_{\text{GB}} = 1/\rho_{\text{GB}}$ as a function of carrier density $n = C|V_G - V_{\text{Dirac}}|/e$ for the two devices shown in Fig. 2, A and B, where C is the gate capacitance per unit area. The GB from growth A was overall an order of magnitude more conductive, as discussed previously. At low carrier concentrations

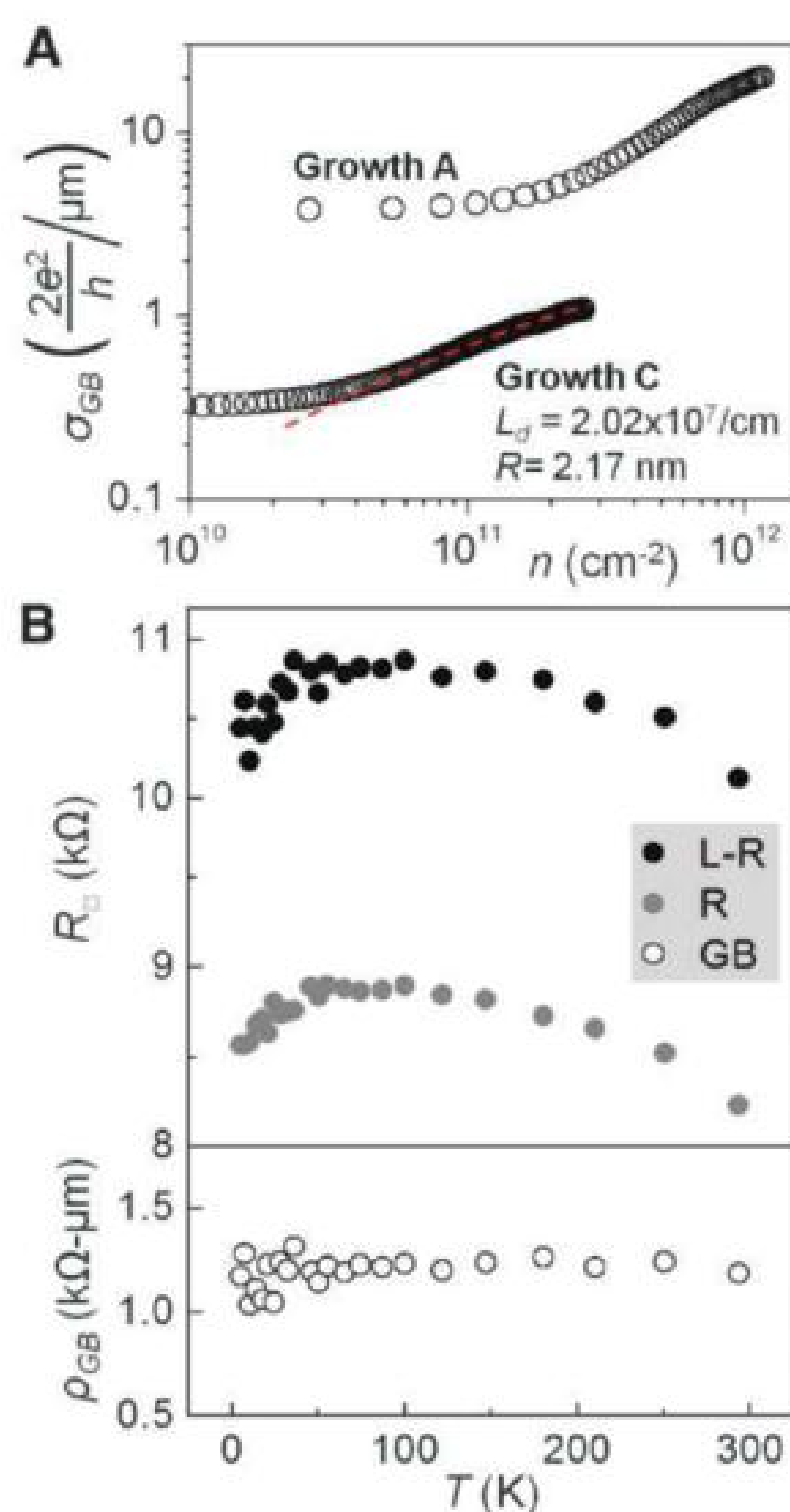


Fig. 3. (A) GB conductance as a function of carrier density from data in Fig. 2, A and B. Growth C data are fit to a model of defect scattering from midgap states. (B) Temperature dependence of inter- (L-R) and intra- (R) domain resistance from growth A device shows that ρ_{GB} is insensitive to temperature.

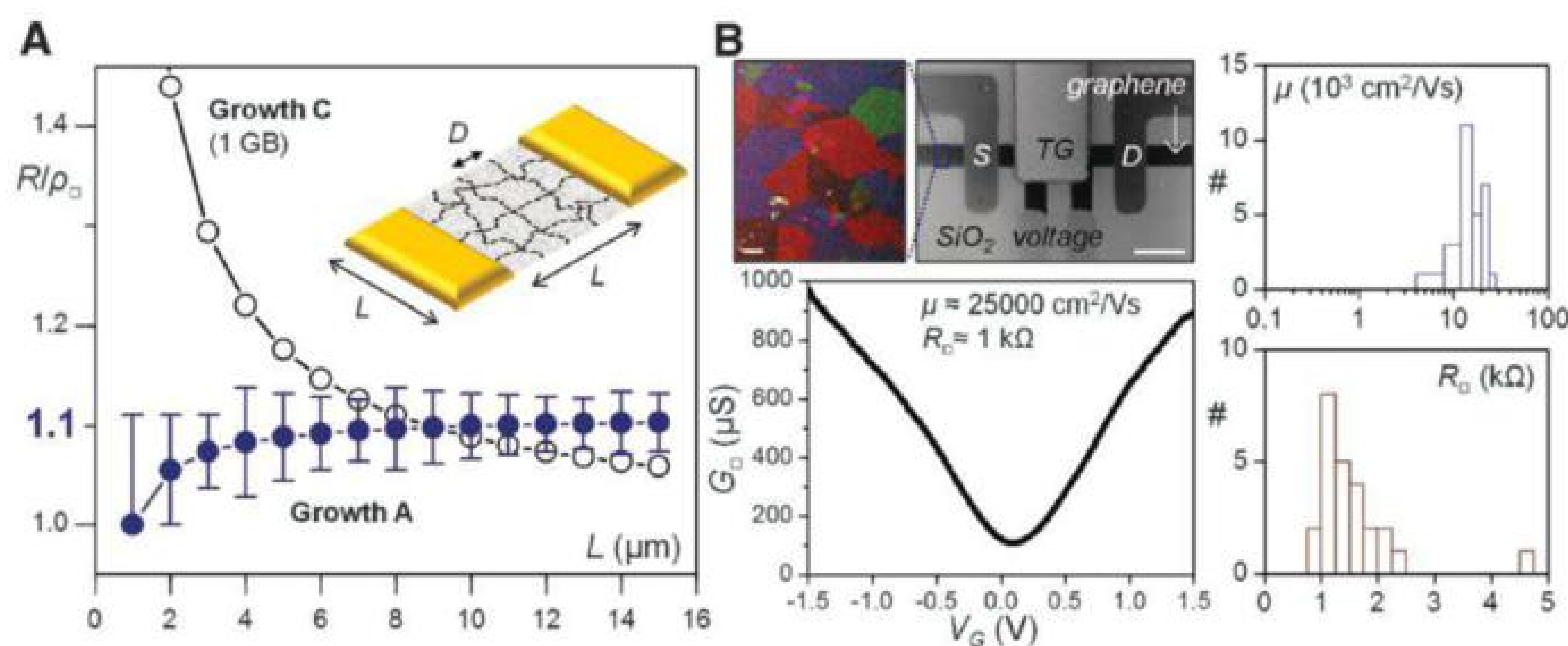


Fig. 4. (A) Model of device resistance as a function of device size for growths A and C, using empirically determined λ and D . Smaller devices with 1 GB from growth C are very resistive, while growth A devices with the statistically expected number of GBs show uniform high performance over a large range of sizes. (B) (Left) Grayscale SEM image (scale bar, 10 μ m) of ≈ 5 by 5 μ m growth A-type graphene device fabricated on SiO₂/Si and representative color DF-TEM image (scale bar, 1 μ m) of domain structure. Transport characteristics demonstrate performance on par with exfoliated graphene. (Right) Histograms for field-effect mobility and p -type sheet resistance of 28 similarly fabricated devices show excellent electrical behavior overall.

near the neutrality point, both values saturated to a minimum as a result of the residual density induced by charge inhomogeneities (16). Away from their minima, σ_{GB} increased slightly sublinearly with n . The increase of GB conductance with doping indicates long-range scattering, because weak point disorder is insensitive to carrier density (17). Currently, both charge-impurity disorder (18) and strong disorder giving rise to mid-gap states (19) are expected to yield conductivity that is roughly linear with carrier density, so it is difficult to untangle these effects a priori. However, the latter predicts increasingly sublinear behavior as the size of the disorder is increased. If we assume that strong disorder dominates the GB resistance in the limit of large ρ_{GB} as from growth C, we can model $\sigma_{\text{GB}} = (2e^2/\pi h)(n/L_d)\ln^2(\sqrt{\pi n} R)$, where L_d is the linear density of defects at the GB and R is the radius of the defect potential. Fitting our data for growth C to this expression away from the conductivity minimum, we obtained $L_d = 2.0 \pm 0.1 \times 10^7 \text{ cm}^{-1}$, $R \approx 2 \text{ nm}$. By comparison, R here was an order of magnitude greater than that modeled from ion-induced single-atom vacancies reported previously (20).

Although defect scattering from GBs was sensitive to carrier density, we expected it to be completely insensitive to the effects of temperature. In Fig. 3B, we show the intradomain (R) and cross-domain (L-R) resistivities for another device (growth A), taken simultaneously as a function of temperature from 5 K to 250 K at p -type doping. We see similar weak temperature dependence for both measurements, which may be the result of impurities in the graphene or on the substrate for this particular sample (21). L-R, however, always appears a fixed value larger throughout, from which we extract a relatively constant GB resistivity of $1 \text{ k}\Omega\text{-}\mu\text{m}$ for the entire temperature range, consistent with our picture of defect-dominated transport.

The results of our TEM and transport measurements on individual GBs in CVD graphene show that the conductance of GBs was highly correlated with their structure. Our data also suggest that it is necessary to control GB connectivity as well as domain size in order to maximize the overall electrical transport properties in polycrystalline graphene. For this, it is essential to develop a systematic understanding of the combined electrical effect of λ (representing GB connectivity) and D (domain size) under various graphene growth conditions and device geometries. Below, we present a simple model based on these two empirically derived parameters that can be used to optimize electrical performance in graphene devices at all length scales.

There are two necessary criteria to uphold in order to successfully integrate CVD graphene into electronic applications. Not only is it important to maximize the performance of individual graphene devices, but achieving uniform performance across many devices is also highly desirable. Because GBs introduce inhomogeneity in the graphene film on the length scale D , a trade-

off occurs for the above two criteria. In the limit where device size $L = W \ll D$, graphene consisting of a single domain will clearly have the best individual performance, as device resistance is $R = \rho_{\square}$. However, devices that cross a GB will have $R = \rho_{\square}(1+\lambda/L)$, which could pose a hindrance if λ is large, such as in growth C. In the opposite limit, where $L \gg D$, the devices see $R = \rho_{\square}(1+n\lambda/L)$, where n is the number of GBs crossed. However, we expect $n \approx L/D$, so all devices will see a uniform $R \approx \rho_{\square}(1+\lambda/D)$, which also may not severely degrade performance if λ is small, such as in growth A. The plot in Fig. 4A captures what is described here quantitatively. Here, we calculated normalized device resistance $R/\rho_{\square}$ as a function of device size L up to $15 \mu\text{m}$ for graphene from the two electrically characterized growths A ($D \approx 1 \mu\text{m}$) and C ($D \approx 50 \mu\text{m}$). The curve in black shows the result of growth C crossing one GB using the empirically obtained $\lambda_C = 880 \text{ nm}$. Normalized resistance is very large at small L and decreases asymptotically to 1, the intrinsic limit without GBs. The curve in blue shows the result of growth A as the device crosses the expected number of GBs $n = L/D - 1$ (the error bars indicate a $\pm\sqrt{n}$ standard deviation from this number, rounded to the nearest integer). Here, resistance is 1 at small L and slowly increases, as calculated by $\lambda_A = 110 \text{ nm}$. The two curves cross at $L \approx 9 \mu\text{m}$. However, even above this length, the growth A sample will not severely degrade in performance as resistance eventually saturates to only 10% ($\approx\lambda/D$) larger than the intrinsic limit for monocrystalline graphene. Similarly, device mobility will approach 90% ($\approx 1 - \lambda/D$) of that of a single crystal. Of equal importance, such devices show uniform performance over a large range of lengths with less likelihood of failure for an individual device.

This analysis suggests that well-stitched GBs are not the dominant scattering mechanism to affect large-scale device transport (22) and points to an exciting potential for the high electrical performance of polycrystalline graphene. Although the synthesis of growth C could, in principle, be further optimized to achieve large domains together with better GB connectivity, it seems that we can already achieve most of the performance capability of single-crystal graphene in large-scale, polycrystalline devices using our current growth conditions (growth A), despite limited domain size.

We have now demonstrated the performance potential of such samples experimentally, as shown in Fig. 4B. We have fabricated a set of ultra-clean graphene devices on oxidized silicon wafers (≈ 5 by $5 \mu\text{m}$, with 100-nm SiO_2 top gates) using growth A-type synthesis from a semiconductor-grade CVD system that is thoroughly helium leak-checked and conventional lithography without the additional imaging steps of TEM. For a particular high-performance device, we achieved a field-effect mobility of $25,000 \text{ cm}^2/\text{Vs}$ and sheet resistance of $\approx 1 \text{ kilohm}$ at a relatively low carrier density ($\sim 3 \times 10^{11} \text{ cm}^{-2}$) (Fig. 4B, bottom left), on par with that of supported, exfoliated graphene

(16). The use of boron nitride as a gate dielectric could perhaps be used to further improve device performance (23, 24). On the right, we plotted statistics across 28 similarly fabricated devices and saw that almost all devices had field-effect mobilities above $10,000 \text{ cm}^2/\text{Vs}$ and resistances below 2 kilohm . Thus, GBs in chemically grown, polycrystalline graphene can be optimized to have a minimal electrical impact on the overall transport properties of the device, in accordance with our model and findings above. We note that our experimental techniques presented here are not limited to the study of graphene but pave the way for electrical studies of other newly reported nanomaterials, such as layered, two-dimensional inorganic compounds and their hybrids (25), together with the imaging power of TEM.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S7
References (26, 27)

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Theory Untangles the High-Resolution Infrared Spectrum of the *ortho*-H₂-CO van der Waals Complex

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Rovibrational spectroscopy of molecules boasts extremely high precision, but its usefulness relies on the assignment of spectral features to corresponding quantum mechanical transitions. In the case of *ortho*-H₂-CO, a weakly bound complex abundant in the interstellar medium (although not yet observed there), the rather complex spectrum has been unexplained for more than a decade. We assigned this spectrum by comparison with a purely ab initio calculation. For most lines, agreement to within 0.01 cm⁻¹ between experiment and theory was achieved. Our results show that the applicability of rovibrational spectroscopy can be extended with the assistance of high-accuracy quantum mechanical computations.

Interpretations of molecular spectra are based on computational models deeply rooted in quantum mechanics, but in most cases the parameters of the models are fitted to observations and do not require any ab initio quantum mechanical input. The measurements then provide highly accurate benchmarks that are used to evaluate the performance of theoretical methods. However, there are also systems wherein rovibrational spectra are so congested and seemingly random that pattern recognition is insufficient for assignment. An example is the infrared absorption spectrum of *ortho*-H₂-CO, first measured (1) in 1998 but until now unassigned. The van der Waals complex of H₂ with CO has been the subject of considerable experimental and theoretical effort during the past three decades, primarily because of its astrophysical importance (2). H₂ is the main component of interstellar molecular clouds but is difficult to detect directly. In its place, CO is used for mapping interstellar matter in galaxies. The interaction between H₂ and CO is therefore highly relevant (3).

The H₂-CO complex exhibits very different spectra depending on the *ortho* or *para* coupling of the nuclear spins in H₂. The *ortho* spin wave function is symmetric, whereas the *para* is antisymmetric with respect to an exchange of nuclei. Because protons are fermions, the total wave function for nuclear motion must be antisymmetric, and thus the *ortho* rotational functions must be antisymmetric (and the *para* rotational functions must be symmetric). This means that the allowed rotational quantum numbers j_1 are odd for *ortho*-H₂ and even for *para*-H₂. In particular, *ortho*-H₂ cannot exist in the lowest $j_1 = 0$ state, whereas *para*-H₂ can. (For D₂, with bosonic nuclei, the situation is reversed.) Because H₂ rotational energies are large relative to the strength

of the H₂-CO interaction, the rovibrational wave functions of the complex are dominated by terms containing only a single H₂ rotational function: $j_1 = 1$ for *ortho*-H₂, and $j_1 = 0$ (a much simpler function) for *para*-H₂. Nuclear symmetry thus renders the *para*-H₂-CO spectrum considerably simpler than that of *ortho*-H₂-CO. The infrared spectrum of *para*-H₂-CO has been recorded and analyzed (1), with subsequent millimeter-wave measurements providing more precise information (4, 5). Similar results (with $j_1 = 0$) are available for *ortho*-D₂-CO (6–8). However, the spectrum of *ortho*-H₂-CO (1) was too complex for a conclusive analysis. Our goal was to assign this spectrum by comparison with a theoretical spectrum based on an accurate ab initio potential energy surface for the H₂-CO interaction.

Theoretical transitions are computed as energy differences between quantum states, which reveals the character of experimental features that can be correlated with the calculated ones. However, for *ortho*-H₂-CO the predictions must be extremely accurate because the spectrum is very congested. We previously tried to assign this spectrum by using successively more accurate potential energy surfaces V_{98} (9) and V_{04} (10). Although these surfaces were successful in predicting other spectra and scattering cross sections, the accuracy produced by V_{98} or V_{04} (about 1 cm⁻¹ or 0.1 cm⁻¹, respectively, for the *para*-H₂-CO spectrum) was insufficient for *ortho*-H₂-CO. Attempts to improve the potentials by tuning them to better fit the *para*-H₂-CO spectrum were not successful.

A decrease of the uncertainty of theoretical predictions below 0.1 cm⁻¹ was not possible within the limits of computer resources in 2004 when V_{04} was obtained, because the applied level of electronic structure theory had to be restricted to the coupled-cluster method with single, double, and noniterative triple excitations [CCSD(T)]. Here, we break this barrier and compute the surface with the inclusion of complete triple and noniterative quadruple excitations [CCSDT(Q)]. We also increase the size of basis sets and use extrapolations to complete basis set limits. Further-

more, we increase the dimensionality of our surface to the full six dimensions, whereas V_{98} was four-dimensional (rigid-monomer approximation for both H₂ and CO) and V_{04} was five-dimensional (rigid-monomer approximation for CO).

We used augmented correlation-consistent basis sets (11), aug-cc-pVXZ, where X is the so-called cardinal number, with bases up to $X = 6$ at the Hartree-Fock level, up to $X = 5$ at the CCSD(T) level, and up to $X = 3$ at the CCSDT(Q) level. We also included a set of 69 midbond functions (12) in some calculations. Extrapolations were used for the CCSD(T) correlation energy contributions only, as the Hartree-Fock energies are sufficiently converged without extrapolations and small post-CCSD(T) terms were computed in basis sets that are not large enough to allow reliable extrapolations. On the basis of the convergence patterns in X , we estimate that our best values of interaction energies have errors below 0.5 cm⁻¹ near the van der Waals minimum. This is substantially better than in our previous potentials (e.g., V_{04} is 1.0 cm⁻¹ different from the present value at the minimum). The post-CCSD(T) excitations are likely the main reason for improvements achieved in the present work, as such excitations are highly anisotropic and contribute a few percent to interaction energies for some configurations, although the increase of the basis size also played a major role.

Direct calculation of spectra from a six-dimensional surface is unnecessarily difficult. Accurate results (much more so than from any rigid-monomer surface) can be obtained by averaging over the monomer vibrations (10, 13–16). We computed the potential on a six-dimensional grid, performed averages eliminating the r (HH) and s (CO) coordinates, and fitted a four-dimensional surface to the averaged data. The averaging was achieved by representing the six-dimensional surface as a second-order Taylor expansion in r and s around some reference points with derivatives computed numerically. The total number of grid points used to obtain the leading term in the Taylor expansion was 1261, whereas six times as many points were needed for calculations of the derivatives in r and s coordinates. We used a step of 0.025 bohr. The four-dimensional sets of averaged interaction energies were fitted by least-squares minimization using the analytic functional form from (9, 17) [see eqs. 11 to 17 in (9)], with van der Waals constants and damping parameters from (9). The asymptotic constants at each inverse power of R were uniformly scaled using only interaction energies with $R \geq 10$ bohr to account for the improved basis sets and higher level of theory relative to (9). The two resulting surfaces, corresponding to CO vibrational states with $v = 0$ and 1, are denoted V_0 and V_1 , respectively. (A FORTRAN program computing these surfaces is available from the authors upon request.)

Rovibrational calculations were performed with the BOUND package (18) using the coupled-channel method within the space-fixed coordi-

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nate formalism. Details of the calculations are the same as in (10). In particular, for *ortho*-H₂ we used the angular basis set taking into account the $j_1 = 1$ and $j_1 = 3$ dependence. Several of the measured lines involve resonances lying above

the dissociation limit, and their identification was a major part of our calculations. The energy zero is taken with *para*-H₂ and CO in their ground states at infinite separation, so that the dissociation threshold for *ortho*-H₂-CO is $E_{\text{thr}}^{\text{ortho}} = 2B =$

118.644 cm^{-1} (i.e., the $j_1 = 1$ energy), where B is the H₂ rotational constant (19). The discrete energy levels above $E_{\text{thr}}^{\text{ortho}}$ represent either resonance or continuum states. The former can be identified by their stability with respect to changes of var-

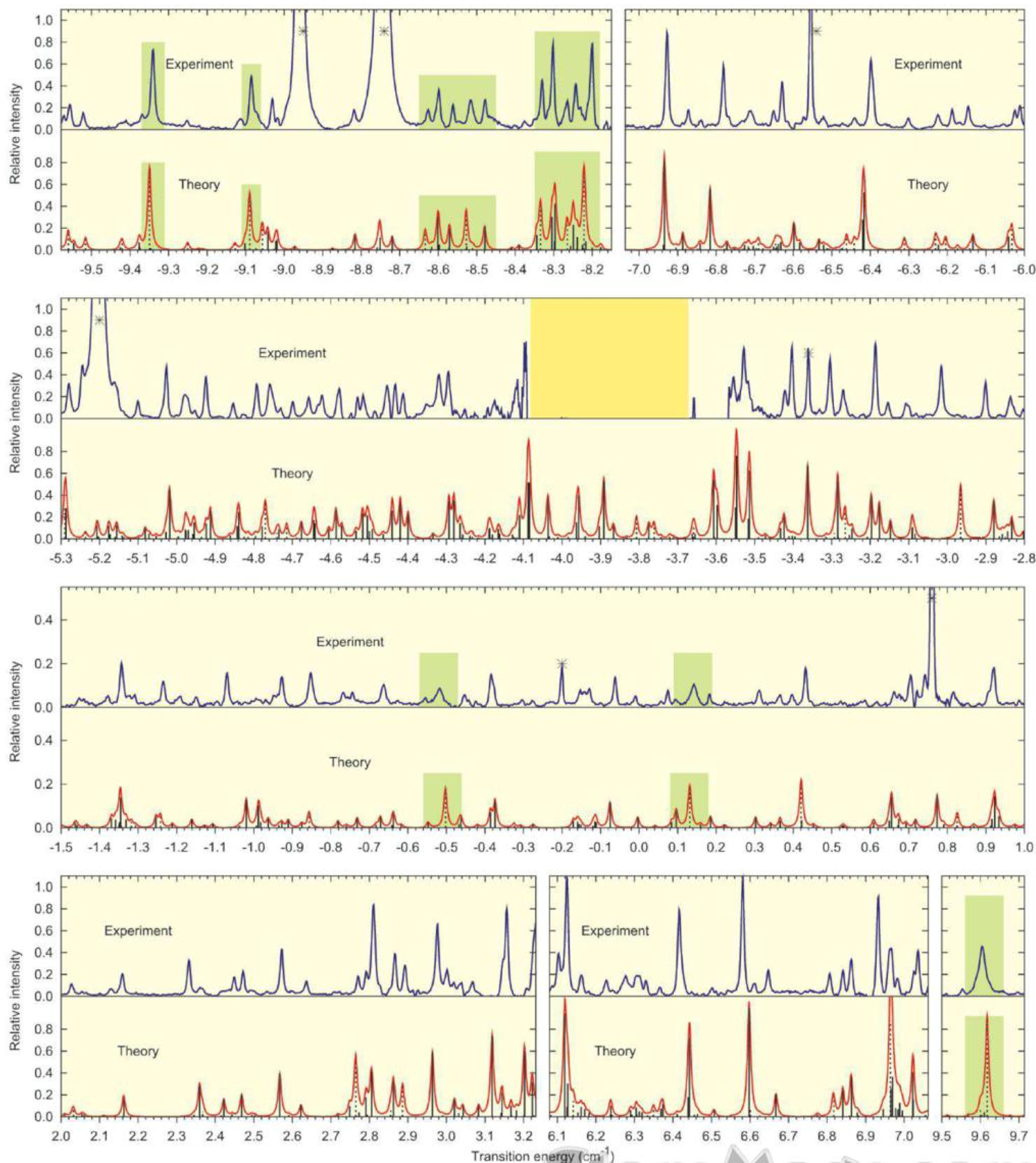


Fig. 1. Comparison of experimental and theoretical infrared spectra of *ortho*-H₂-CO. The theoretical spectra are calculated from the V_0 and V_1 surfaces at $T = 49 \text{ K}$. The strongest calculated transition is set to an intensity of 1; only transitions stronger than 0.01 are shown. Solid lines indicate calculated transitions between two bound

states, whereas dashed lines are used if at least one of the states is a resonance. Asterisks indicate CO monomer lines. Transition energies are given relative to the transition energy of 2143.272 cm^{-1} , the $\nu = 1 \leftarrow 0$ vibrational frequency of the isolated CO monomer. The shaded regions are discussed in the text.

ious parameters in our calculations—for example, boundary conditions or angular basis set size. The assignment of spectra would not be possible without the use of theoretical intensities. These were computed using programs developed in (20).

We first computed the *para*-H₂-CO spectrum (table S1). The root mean square error (RMSE) discrepancies between the energy levels deduced from experiment (1) and those computed here are 0.006 cm⁻¹ and 0.007 cm⁻¹ for $\nu = 0$ and 1, respectively, on the 86 levels included in this comparison (the energies are relative to the ground-state level in both cases). Individual discrepancies vary between -0.011 and +0.013 cm⁻¹ for $\nu = 0$ and between -0.015 and +0.009 cm⁻¹ for $\nu = 1$. The best previous theoretical results (10) had an RMSE of 0.05 cm⁻¹ for $\nu = 0$. Direct comparison of the theoretical and experimental *para*-H₂-CO spectra including calculated intensities (fig. S1) showed results in extremely

good agreement. However, we must anticipate larger discrepancies for *ortho*-H₂-CO due to the nature of its rovibrational states, which are more sensitive to local inaccuracies in the potential energy surface.

The experimental *ortho*-H₂-CO spectrum was observed using a long-path, low-temperature (200 m, 49 K) absorption cell coupled to a Fourier transform infrared spectrometer, as described in (1). Data used here were obtained with a spectral resolution of 0.005 cm⁻¹, CO partial pressure of about 0.3 torr, and normal H₂ partial pressure of 3.2 torr. The raw absorption spectrum was flattened (by removing the varying background due to CO monomer absorption) and converted to absorbance (1). Normal H₂ contains 75% *ortho*-H₂ and 25% *para*-H₂. To remove the *para*-H₂-CO contribution, we measured a spectrum using nearly pure (>99%) *para*-H₂ (1), scaled it appropriately, and subtracted it from the normal H₂ spectrum.

Table 1. Calculated values of *ortho*-H₂-CO rovibrational energies. The $\mathcal{E}_\nu$ and E_ν energies are calculated for the $\nu = 0$ and $\nu = 1$ vibrational states of CO. $\mathcal{E}_\nu$ values are measured relative to the dissociation limit of the ground state of H₂-CO, with H₂ in its ground rotational state, $j_1 = 0$. Because the dissociation limit for $j_1 = 1$ is at 118.644 cm⁻¹, all the energies are positive, with the ground states at 97.967 cm⁻¹ and 97.789 cm⁻¹ for $\nu = 0$ and 1, respectively. E_ν values are given relative to these two states. The only good quantum numbers are the overall angular momentum J and parity $P = e$ or f . The CO rotational quantum number j_2 , the number j_{12} describing the coupling of j_1 and j_2 , and the end-over-end number l are all approximate. $n_{J,P}$ simply numbers consecutive states for each J, P .

$n_{J,P}$	j_2	j_{12}	l	$\lambda(\Lambda)$	$\mathcal{E}_0$ (cm ⁻¹)	E_0 (cm ⁻¹)	$\lambda(\Lambda)$	$\mathcal{E}_1$ (cm ⁻¹)	E_1 (cm ⁻¹)
$J^P = 0^f$									
1	1	1	1	0.97	103.661	5.694	0.97	103.482	5.693
2	2	2	2	0.94	116.929	18.962	0.94	116.659	18.870
$J^P = 0^e$									
1	0	1	1	0.85	98.354	0.387	0.85	98.145	0.356
2	1	0	0	0.18	99.138	1.171	0.20	99.016	1.226
	1	2	2	0.77			0.73		
3	1	0	0	0.73	102.289	4.322	0.72	102.114	4.325
	1	2	2	0.18			0.19		
$J^P = 1^f$									
1	0	1	1	0.86	98.786	0.819	0.85	98.634	0.844
	2	3	3	0.10			0.11		
2	1	1	0	0.90	102.306	4.339	0.89	102.105	4.316
3	1	1	2	0.92	105.543	7.576	0.92	105.355	7.566
4	2	1	1	0.28	109.523	11.556	0.28	109.263	11.474
	2	2	1	0.38			0.37		
	1	2	2				0.10		
	2	3	3	0.12			0.12		
$J^P = 1^e$									
1	0	1	0	0.89	97.967	0.000	0.89	97.789	0.000
2	1	0	1	0.11	99.653	1.686	0.10	99.560	1.771
	1	2	1	0.39			0.46		
	0	1	2	0.17			0.13		
	1	2	3	0.25			0.25		
3	0	1	2	0.63	100.003	2.036	0.66	99.793	2.004
	1	2	1	0.13					
	2	3	2	0.10					
4	1	0	1	0.41	102.822	4.854	0.39	102.619	4.830
	1	1	1	0.44			0.45		
5	1	0	1	0.38	103.541	5.573	0.39	103.352	5.563
	1	1	1	0.43			0.42		
6	2	1	0	0.60	109.545	11.578	0.59	109.284	11.495
	2	2	2	0.19			0.18		

The result (Fig. 1) should be a good approximation of the spectrum of pure *ortho*-H₂-CO. There are gaps in the spectrum corresponding to regions very close to strong CO monomer transitions, and also a few lines (asterisks in Fig. 1) due to various CO monomer isotopologues in natural abundance.

The theoretical *ortho*-H₂-CO spectrum was obtained from the rovibrational energy levels for the $\nu = 0$ and $\nu = 1$ states of CO and the calculated transition intensities. The search for resonance states extended up to an energy of 130 cm⁻¹ (i.e., 11.356 cm⁻¹ above $E_{\text{thr}}^{\text{ortho}}$). The rigorous quantum numbers describing the energy levels are parity P (either e or f) and total rotational angular momentum J . To further determine the characteristics of the states, we computed coefficients $\lambda(\Lambda)$, defined as the ratio of the squared radial factor for a given set of angular (pseudo)quantum numbers Λ to the sum of these squared factors over the whole angular basis set. The results of those calculations for a subset of levels are listed in Table 1; complete results are in table S2. Only contributions from angular basis set functions with $\lambda(\Lambda) > 0.1$ are listed. For many states, there is more than one component with a substantial contribution to the wave function.

The experimental and theoretical spectra are compared in Fig. 1 for selected frequency ranges, with complete results given in fig. S2. Theoretical transitions were broadened using a Lorentzian function with a fixed width obtained by fitting a selected set of well-isolated experimental lines assigned as bound-to-bound transitions. The similarity between the theoretical and measured spectra is striking. In most cases, the accuracy of the theoretical spectrum is high enough to assign experimental counterparts unambiguously. Virtually all strong lines from the experimental spectrum have theoretical counterparts, although in a few cases the spectrum is too dense to make unambiguous matches. The complete assignments are presented in table S3. The largest discrepancy between experimental and theoretical transition energies amounts to 0.06 cm⁻¹, in moderate agreement with the crude estimate of 0.02 cm⁻¹ that can be made on the basis of the *para*-H₂-CO results presented earlier.

In addition to the direct assignment of measured lines, several other aspects of theoretical work provide a better understanding of the spectrum. First, theory covers all spectral regions, including those not accessible to experiment because of strong CO monomer absorption (e.g., the range -4.1 to -3.7 cm⁻¹ in Fig. 1). Second, theory clearly distinguishes bound-to-bound state transitions from those involving resonance states. This distinction is critical for experimental determination of the dissociation energy. There are several strong lines in Fig. 1 that are well separated from other lines and not much broader than typical lines (e.g., lines at -9.34 and -9.08 cm⁻¹), so that only comparisons with theory show that these transitions involve resonance states. Another interesting feature can be found in the region from -8.64 to -8.47 cm⁻¹, composed

of five strong lines. Two of these are resonance transitions, and if such lines were missing from the theoretical spectrum, an assignment of this region would be difficult. Note that the theoretical and experimental intensities of the discussed lines match well except for the peak at -8.53 cm^{-1} , which appears weaker in the observed spectrum. However, the discrepancy is only apparent because the experimental line is clearly broader, indicating a relatively wide resonance state. If the theoretical line were appropriately broadened, the heights would agree much better, but as noted above, a fixed broadening function was used for all theoretical lines, including resonance ones. (In principle, theory can provide these resonance widths, but this application is beyond the scope of the present work.)

Another example of how theory elucidated the spectra is manifested in the analysis of two wide observed peaks at -0.52 and $+0.14\text{ cm}^{-1}$, and of a very strong one at 9.61 cm^{-1} , the shapes of which could suggest superpositions of several weak lines. With theoretical insight, we can attribute this shape to their resonance character. On the other hand, there may be uncertainty in theoretical approach about whether a given level above the dissociation limit is a resonance or continuum state, and the presence of broadening in the experiment confirms their resonance character. Such

experimentally confirmed resonances are marked with daggers in table S2.

We have assigned an experimental infrared spectrum of the *ortho*-H₂-CO complex in the region of the CO fundamental band. Also, theory allowed us to better understand some subtle aspects of the experimental spectrum. On the other hand, the experimental precision of about 0.001 cm^{-1} provides a challenging test to evaluate the quality of theory and the adequacy of various approximations. For example, our work provides further confirmation that surfaces averaged over intramonomer motion are sufficiently accurate for applications such as the present one. The assigned spectrum and the calculated potential surface can now be used to interpret astronomical observations and to enable precise millimeter-wave measurements that need infrared guidance because of their limited frequency range scanning capabilities.

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Supplementary Materials

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Tables S1 to S3
Figs. S1 and S2

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Secreted Kinase Phosphorylates Extracellular Proteins That Regulate Biomineralization

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Protein phosphorylation is a fundamental mechanism regulating nearly every aspect of cellular life. Several secreted proteins are phosphorylated, but the kinases responsible are unknown. We identified a family of atypical protein kinases that localize within the Golgi apparatus and are secreted. Fam20C appears to be the Golgi casein kinase that phosphorylates secretory pathway proteins within S-x-E motifs. Fam20C phosphorylates the caseins and several secreted proteins implicated in biomineralization, including the small integrin-binding ligand, N-linked glycoproteins (SIBLINGs). Consequently, mutations in Fam20C cause an osteosclerotic bone dysplasia in humans known as Raine syndrome. Fam20C is thus a protein kinase dedicated to the phosphorylation of extracellular proteins.

Protein phosphorylation is a nearly universal mechanism used by cells to regulate intracellular and extracellular processes (1). The majority of phosphoproteins are intracellular; however, numerous extracellular proteins are phosphorylated (2–4). The first evidence of pro-

tein phosphorylation was in 1883, when the secreted protein casein was shown to contain stoichiometric amounts of phosphate (5). Casein has been used as a model substrate for the detection of several protein kinases, including the first discovery of a protein kinase activity and the identification of casein kinase-1 and casein kinase-2 (6, 7). However, these enzymes are physiologically unrelated to casein because they are mainly cytosolic and nuclear proteins that would be unlikely to encounter casein in the secretory pathway and have therefore been renamed protein kinase CK1 and protein kinase CK2 (6). A

physiological casein kinase activity has been characterized from highly enriched Golgi fractions of lactating mammary gland, liver, brain, and kidney and named Golgi-enriched fraction casein kinase (GEF-CK) (8–10). The GEF-CK specifically recognizes the consensus S-x-E/pS (where x is any amino acid and E/pS can be Glu or phosphoserine) (11). This motif is phosphorylated in some 75% of human plasma and cerebrospinal fluid phosphoproteins (2–4, 12).

To identify candidates for the GEF-CK, we searched for protein kinases containing a signal peptide (SP) and no transmembrane helix. This architecture would orient the kinase in the lumen of the Golgi, in close proximity to proteins destined for secretion. Four-jointed is one such kinase that localizes within the Golgi and phosphorylates extracellular domains of cadherins in *Drosophila* (13). Therefore, we used the human four-jointed (Fjx1) sequence to identify, by means of Position-Specific Iterated- Basic Local Alignment Search Tool (PSI-BLAST), a family of eukaryotic proteins that are distantly related to the bacterial kinase HipA (Fig. 1A and fig. S1) (14). Members of the family with sequence similarity 20 (Fam20) and 198 (Fam198) have SPs and conserved residues required for protein kinase activity (fig. S2). To determine whether these proteins were secreted, we expressed C-terminal FLAG-tagged proteins in the human osteosarcoma cell line U2OS and analyzed FLAG immunoprecipitates from cell extracts and conditioned medium by means of protein immunoblotting. Some 90% of Fam20C was detected in the medium, and the

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intracellular protein colocalized with the Golgi resident protein GM130 (Fig. 1, B and C). Similarly, the other family members localized within the secretory pathway, and most were secreted (fig. S3, A and B). To test whether Fam20C was a protein kinase, we generated a human embryonic kidney (HEK) 293 T cell line stably expressing a C-terminal FLAG-tagged Fam20C and immunopurified the fusion protein to homogeneity from conditioned medium (Fig. 1D). The recombinant protein was N-linked glycosylated (fig. S4) and catalyzed phosphorylation of several peptides when incubated with [γ - 32 P]ATP (ATP, adenosine 5'-triphosphate) and a Ser-Thr kinase substrate array (Fig. 1E). Many of the substrates contained an S-x-E motif (Fig. 1F). Therefore, we synthesized a substrate array consisting of peptides representing phosphorylation sites from secreted proteins (table S1) (2, 3, 12). Fam20C phosphorylated ~55% of the peptides and had

preference for peptides containing S-x-E motifs (fig. S5, A and B, and table S1). Fam20C phosphorylated Ser more readily than Thr and Tyr and tolerated only Glu at the $n+2$ position (fig. S5, C and D). Thus, Fam20C is a secreted protein kinase that specifically recognizes the consensus sequence S-x-E.

A peptide substrate reproducing one of the sites phosphorylated in β -casein, KKIEKFQSE-EQQQ (β 28-40), is selectively phosphorylated by GEF-CK (11). The GEF-CK consensus site partially overlaps with that of the polo-like kinase-2/3 (PLK-2/3) consensus site, E-x-x-S-x-E. However, β 28-40 is not phosphorylated by PLK2 (15). Furthermore, the GEF-CK requires Mn^{2+} , as an alternative to Mg^{2+} as the activating cation (10, 16). Fam20C phosphorylated β 28-40 in the presence of $MnCl_2$ and $CoCl_2$ more efficiently than in the presence of $MgCl_2$ and $CaCl_2$ (Fig. 2A). GEF-CK is insensitive to the kinase inhibitor staurosporine

(10). Staurosporine (200 μ M) had no effect on Fam20C activity (fig. S6). Fam20C phosphorylated recombinant β -casein in a time-dependent manner, whereas the catalytically inactive D478A mutant, that is unable to coordinate Mn^{2+} , did not (Fig. 2B and fig. S2). (In the mutants, other amino acids were substituted at certain locations; for example, D478A indicates that aspartic acid at position 478 was replaced by alanine). Protein immunoblotting of immunoprecipitates from conditioned medium of U2OS cells overexpressing V5-tagged α_{s1} -casein with FLAG-tagged Fam20C revealed a mobility shift in α_{s1} -casein that was absent when α_{s1} -casein was coexpressed with the inactive Fam20C D478A mutant (Fig. 2C). Treatment with λ -phosphatase reversed the Fam20C-dependent mobility shift of α_{s1} -casein, confirming a phosphorylation event (Fig. 2C). Furthermore, depletion of endogenous Fam20C in HEK293 T cells with small interfering RNA

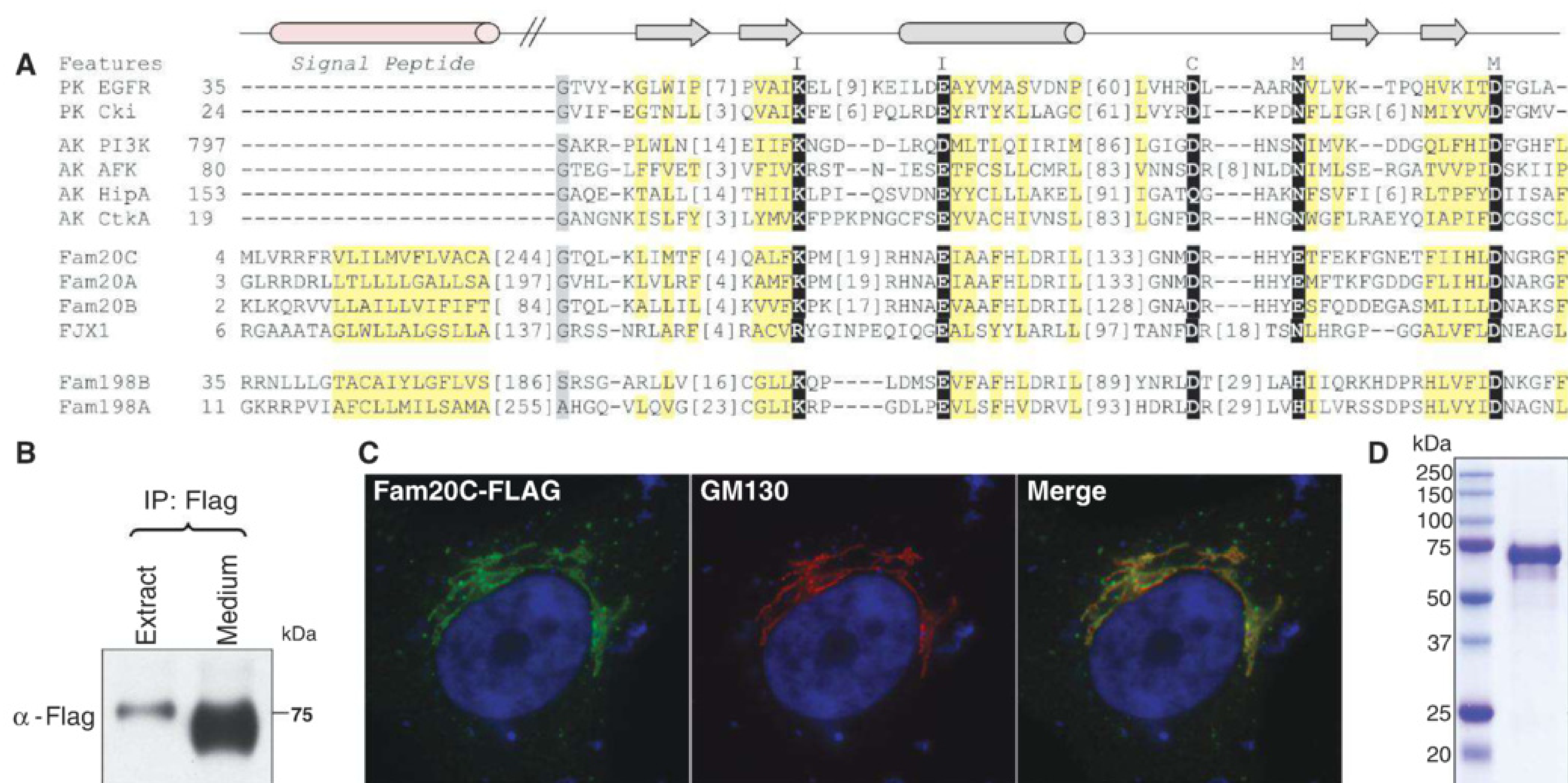


Fig. 1. Fam20C is a secreted protein kinase that phosphorylates S-x-E motifs. (A) Sequence alignment of members of the Fjx1 family of proteins with Canonical (PK) and atypical (AK) protein kinases highlighting conserved residues required for protein kinase activity (black), hydrophobic residues contributing to the structure (yellow), and the SP. Conserved protein kinase secondary structural elements and sequence features are indicated above. I, ion pair residues; C, catalytic residue; M, metal-binding residues. (Single letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.) (B) Protein immunoblotting of FLAG immunoprecipitates from the cell extract and conditioned medium of U2OS cells expressing FLAG-tagged Fam20C. (C) Immunofluorescence analysis of HeLa cells overexpressing FLAG-tagged Fam20C. The Golgi resident protein GM130 is shown. (D) SDS-polyacrylamide gel electrophoresis

(SDS-PAGE) and Coomassie staining of FLAG-tagged Fam20C immunopurified from the conditioned medium from HEK293 T cells. (E) Autoradiograph of a kinase substrate array depicting peptides (in duplicate) phosphorylated by Fam20C. (F) Sequences of peptides phosphorylated by Fam20C in (E).

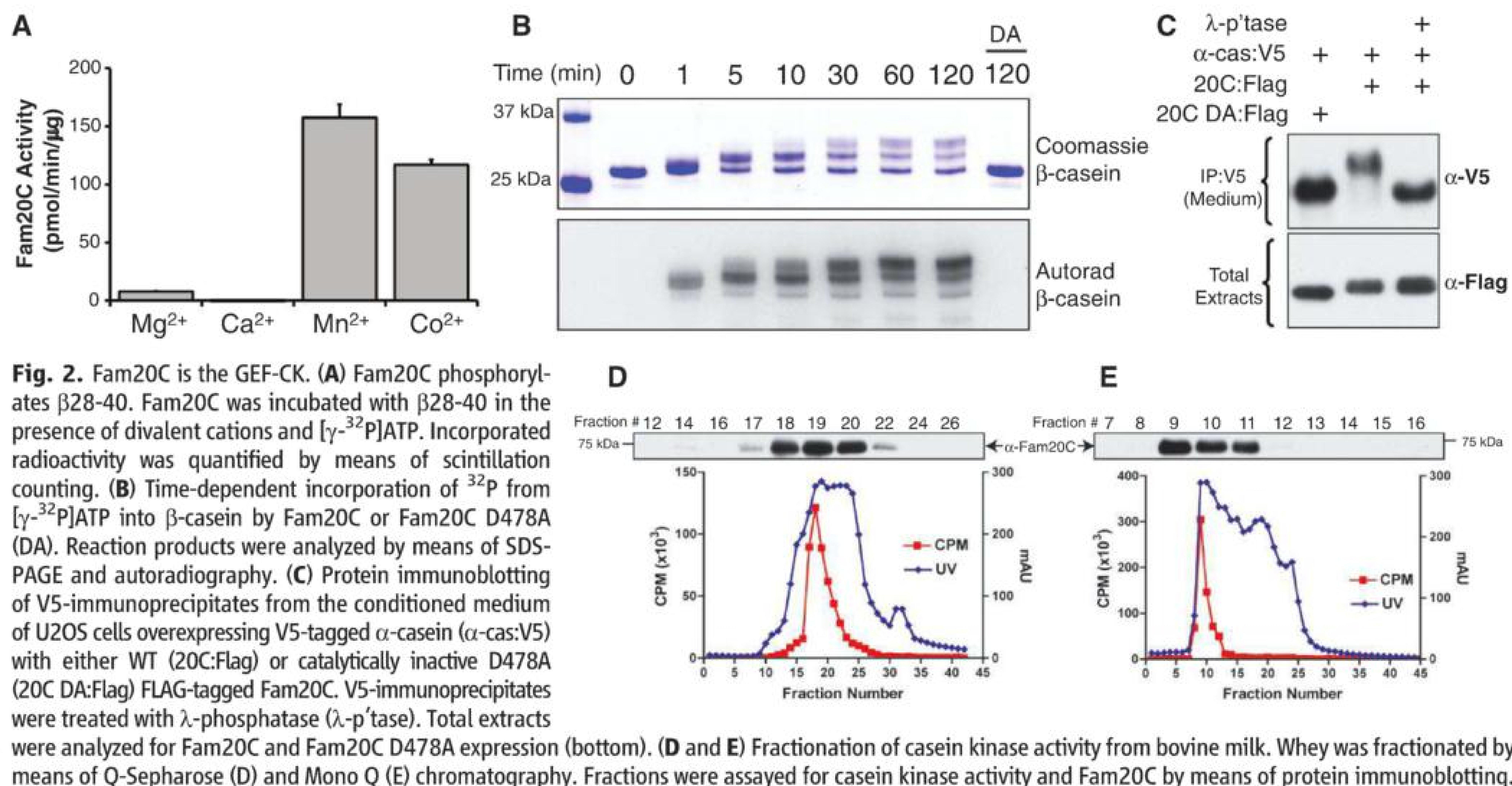
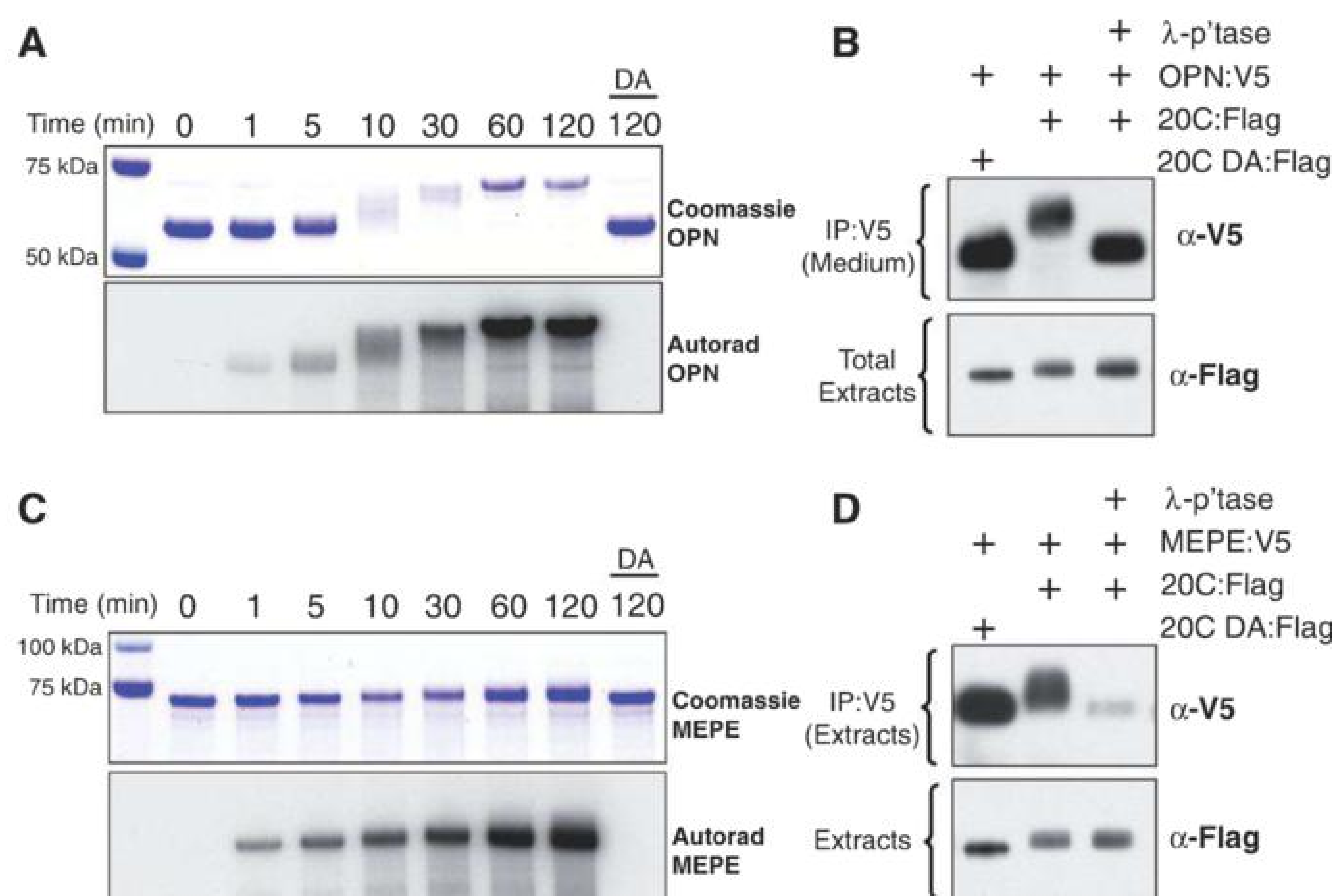


Fig. 2. Fam20C is the GEF-CK. **(A)** Fam20C phosphorylates β28-40. Fam20C was incubated with β28-40 in the presence of divalent cations and [γ -³²P]ATP. Incorporated radioactivity was quantified by means of scintillation counting. **(B)** Time-dependent incorporation of ³²P from [γ -³²P]ATP into β-casein by Fam20C or Fam20C D478A (DA). Reaction products were analyzed by means of SDS-PAGE and autoradiography. **(C)** Protein immunoblotting of V5-immunoprecipitates from the conditioned medium of U2OS cells overexpressing V5-tagged α-casein (α-cas:V5) with either WT (20C:Flag) or catalytically inactive D478A (20C DA:Flag) FLAG-tagged Fam20C. V5-immunoprecipitates were treated with λ-phosphatase (λ-p'tase). Total extracts were analyzed for Fam20C and Fam20C D478A expression (bottom). **(D and E)** Fractionation of casein kinase activity from bovine milk. Whey was fractionated by means of Q-Sepharose (D) and Mono Q (E) chromatography. Fractions were assayed for casein kinase activity and Fam20C by means of protein immunoblotting.

Fig. 3. Phosphorylation of SIBLINGs by Fam20C. **(A)** Time-dependent incorporation of ³²P into OPN by Fam20C or the D478A mutant. Reaction products were analyzed by means of SDS-PAGE and autoradiography. **(B)** Phosphorylation of OPN by Fam20C. Protein immunoblotting of V5-immunoprecipitates from the medium of U2OS cells overexpressing V5-tagged OPN (OPN:V5) with either WT (20C:Flag) or catalytically inactive D478A (20C DA:Flag) FLAG-tagged Fam20C. V5-immunoprecipitates were treated with λ-phosphatase (λ-p'tase). Total extracts were analyzed for Fam20C and Fam20C D478A expression (bottom). **(C)** Phosphorylation of MEPE by Fam20C as in (A). **(D)** Protein immunoblotting of V5-immunoprecipitates from cell extracts of U2OS cells overexpressing V5-tagged MEPE (MEPE:V5) with either WT (20C:Flag) or catalytically inactive D478A (20C DA:Flag) FLAG-tagged Fam20C. Samples were analyzed as in (B).



increased the mobility of overexpressed α_{s1}-casein (fig. S7, A and B). Thus, Fam20C appears to phosphorylate casein in vitro and in mammalian cells.

GEF-CK is secreted and has been purified ~80,000-fold from bovine milk (17). We therefore prepared whey from nonpasteurized, non-homogenized bovine milk and fractionated it by means of Q-Sepharose and Mono Q chromatography (GE Healthcare, Pittsburgh, PA). The resulting fractions were assayed for casein kinase activity and Fam20C protein by means of immunoblotting. Casein kinase activity was detected

primarily in fractions containing Fam20C protein (Fig. 2, D and E). These results support our conclusion that Fam20C is the GEF-CK.

The Fam20C S-x-E consensus motif is found in several secreted proteins, including a family of secretory calcium-binding phosphoproteins (SCPP) that cluster to chromosome 4 in humans (fig. S8) (18). Members of the SCPP family (including the caseins) are secreted phosphoproteins, have a high affinity for calcium, and regulate biomineralization. The small integrin-binding ligand, N-linked glycoproteins (SIBLINGs) are SCPPs encoded by five identically oriented tandem genes

clustered within an ~375-kb span of nucleotides on human chromosome 4 (fig. S8). The genes encode osteopontin (OPN), dentin matrix protein-1 (DMP1), bone sialoprotein (BSP), matrix extracellular phosphoglycoprotein (MEPE), and dentin sialophosphoprotein (DSPP). The SIBLINGs are highly phosphorylated proteins (DSPP has ~200 pSer) and contain multiple phosphorylated S-x-E/S motifs (fig. S9). To explore whether the SIBLINGs are substrates for Fam20C, we purified recombinant OPN, MEPE, and DMP1 from *Escherichia coli* and used them in in vitro kinase assays. Fam20C phosphorylated OPN,

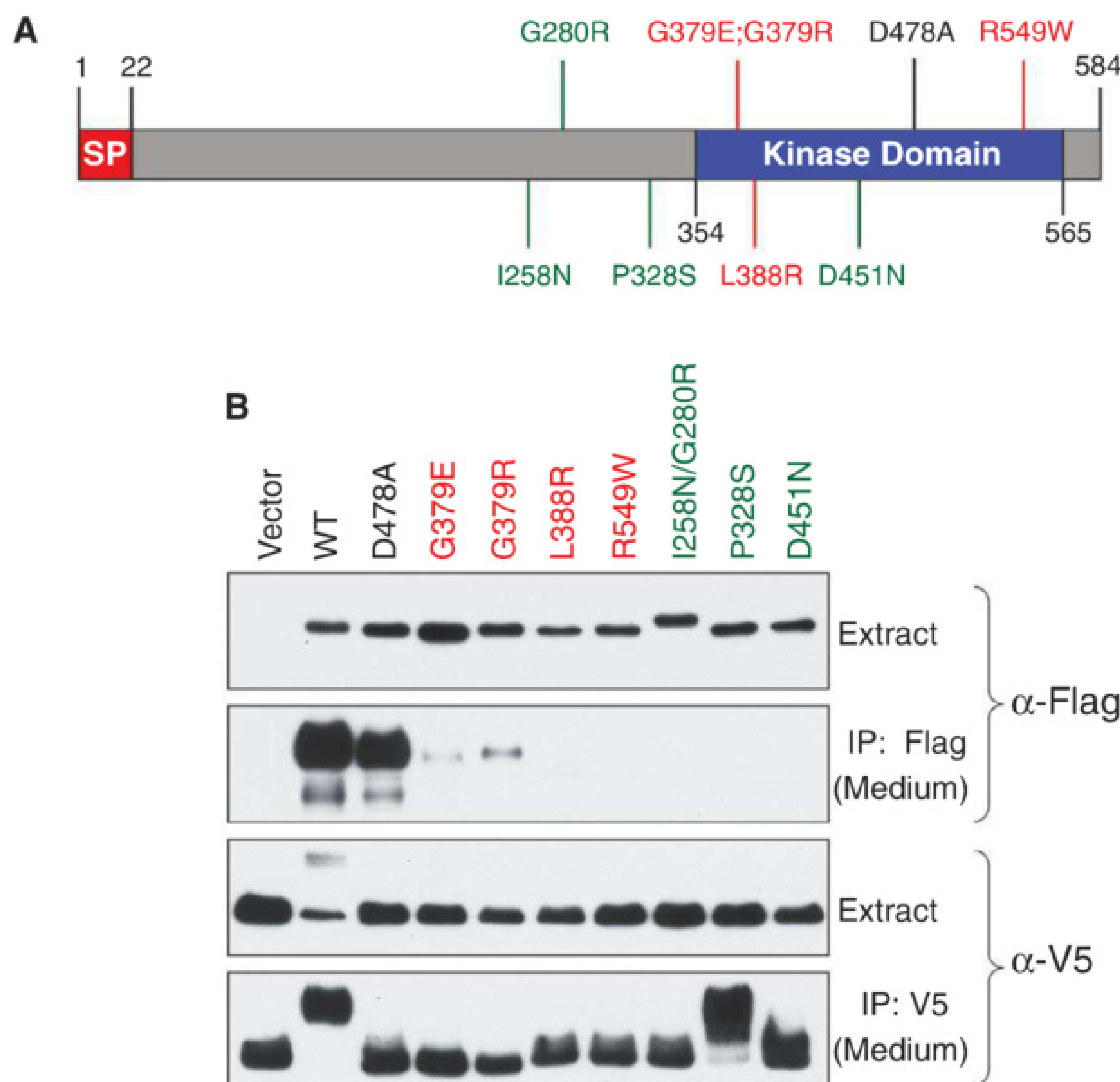


Fig. 4. Fam20C mutations and Raine syndrome. **(A)** Schematic representation of the Fam20C protein depicting missense mutations associated with Raine syndrome. SP, signal peptide. Lethal and nonlethal mutants are in red and green, respectively. **(B)** Secretion of Fam20C mutants. Protein immunoblotting of FLAG and V5 immunoprecipitates from conditioned medium of U2OS cells cotransfected with V5-tagged OPN and FLAG-tagged mutants of Fam20C. Cell extracts were also analyzed for intracellular protein levels.

MEPE, and DMP1 in a time-dependent manner, whereas the inactive Fam20C D478A mutant did not (Fig. 3, A and C, and fig. S10A). Overexpression of V5-tagged OPN or V5-tagged MEPE with FLAG-tagged Fam20C and subsequent protein immunoblotting of immunoprecipitates revealed a mobility shift of tagged OPN and MEPE that was sensitive to λ -phosphatase and absent in the presence of Fam20C D478A mutant (Fig. 3, B and D). Fam20C activity was dependent on a functional SP. Deletion of the SP prevented OPN phosphorylation and Fam20C secretion (fig. S11, A and B). Depletion of Fam20C by using lentiviral-based short hairpin RNA also prevented OPN phosphorylation (fig. S12). Extracellular substrates for Fam20C are not restricted to the SPP family of proteins. Salivary acidic proline-rich phosphoprotein-1 (PRP1)—a secreted phosphoprotein found in saliva and bone morphogenic protein-15 (BMP15), which is a transforming growth factor- β superfamily member secreted by oocytes—were effectively phosphorylated by overexpressed Fam20C but not by the D478A Fam20C mutant (fig. S10, B and C). Consistently, OPN, PRP1, and BMP15 are known substrates of GEF-CK (19–21).

Mutations in Fam20C cause Raine syndrome, a bone dysplasia characterized by osteosclerosis and ectopic calcifications that often causes death

in the neonatal period (22, 23). We reasoned that abnormal phosphorylation of the SIBLINGs might account for the biomineralization phenotype in Raine patients. Phosphorylated S-x-E motifs bind calcium and regulate calcium phosphate precipitation as hydroxyapatite (HA) (24). For example, OPN inhibits HA formation, and this inhibition is dependent on the degree of phosphorylation (25). We therefore generated Fam20C missense mutants associated with lethal and nonlethal forms of Raine syndrome (Fig. 4A). The FLAG-tagged mutants were overexpressed with V5-tagged OPN in U2OS cells. Fam20C secretion and OPN phosphorylation were monitored by means of protein immunoblotting. The Fam20C mutants phosphorylated OPN less efficiently than the wild-type (WT) protein, as judged by the OPN mobility shift (Fig. 4B), even though several mutants colocalized with OPN (fig. S13). Most mutations prevented Fam20C secretion, despite the fact that many localized within the secretory pathway (Fig. 4B and fig. S14). The nonlethal P328S and D451N mutants phosphorylated OPN, albeit not as efficiently as the WT protein. Thus, mutations in Fam20C resulting in Raine syndrome appear to affect Fam20C kinase activity and secretion.

We identified a family of secreted protein kinases and identified Fam20C as the GEF-CK that phosphorylates proteins destined for secretion on

S-x-E (fig. S15). This may have broad biological consequences because S-x-E motifs are phosphorylated in extracellular proteins, including pepsin (26) and fibrinogen (27), as well as some biologically active peptide hormones, including adrenocorticotropin (28), progesterin (29), and many others.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S15

Tables S1 and S2

References (30–41)

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Evolution of a Vertebrate Social Decision-Making Network

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Animals evaluate and respond to their social environment with adaptive decisions. Revealing the neural mechanisms of such decisions is a major goal in biology. We analyzed expression profiles for 10 neurochemical genes across 12 brain regions important for decision-making in 88 species representing five vertebrate lineages. We found that behaviorally relevant brain regions are remarkably conserved over 450 million years of evolution. We also find evidence that different brain regions have experienced different selection pressures, because spatial distribution of neuroendocrine ligands are more flexible than their receptors across vertebrates. Our analysis suggests that the diversity of social behavior in vertebrates can be explained, in part, by variations on a theme of conserved neural and gene expression networks.

Animals have evolved flexible strategies that allow them to respond to their social environment by integrating the salience of an external stimulus with internal physiological cues into an adaptive behavioral response (1, 2). Although individual fitness depends on displaying adaptive behavior patterns (e.g., reproductive or aggressive behavior) in a context-appropriate manner, it has been difficult to examine the evolution of underlying neural and molecular mechanisms because of diversity in ecology, alternative behavior tactics, and brain structure across vertebrates (3, 4). At the same time, this variation provides a unique opportunity to determine the extent to which variance in neural gene expression underlies behavioral adaptations within and across species. To better understand the large-scale patterns of neurochemical evolution across vertebrates, we analyzed the expression profiles for 10 gene products across 12 brain regions that encode and process environmental and physiological cues important for decision-making in 88 species representing five major vertebrate lineages.

Our analysis of the neurochemical evolution of the vertebrate brain focuses on two neural circuits originally described in mammals and since expanded to other vertebrate lineages (5): (i) the social behavior network, which in concert with sex steroid and neuropeptide hormones regulates social behavior such as reproduction, aggression, and parental care (6, 7), and (ii) the mesolimbic reward system, which is generally assumed to evaluate stimulus salience via dopaminergic signaling (8, 9). Although the evolutionary antecedents of these neural circuits (especially regions of the basal ganglia and limbic system) have been debated for the past century (10), it is currently recognized that they were already present in the common ancestor of sarcopterygians (including

tetrapods) and actinopterygians (ray-finned fishes) (5, 7, 11). These two circuits constitute a larger integrated social decision-making (SDM) network that governs stimulus evaluation and behavior across vertebrates (1, 5). Within this framework, we chose to examine the distribution of behaviorally relevant gene products associated with the dopaminergic system [tyrosine hydroxylase (TH), dopamine D1 receptor, or DARPP-32], sex steroid hormone signaling (aromatase and nuclear receptors for estrogen, androgen, and progesterone), and nonapeptide systems (vasopressin, oxytocin, and their receptors). These pathways play fundamental roles in regulating complex social behavior in all vertebrate taxa (12, 13); however, we excluded several neurochemical systems of obvious importance, such as other aminergic, opioid, and neuropeptide pathways, as well as the D2-like dopamine receptor, because of a lack of comprehensive information available across taxa.

To compare the neurochemical profiles of brain regions across vertebrates, we analyzed decades of research in vertebrate neurochemistry by examining published neuroanatomical micrographs presenting mRNA or protein expression. Within each species, we first ascertained whether a gene product of interest was present or absent in SDM network nodes (Fig. 1) and then determined the consensus for that vertebrate lineage. Some brain regions given mammalian names in our analysis here may not represent discrete homologs of mammalian structures (5). Additionally, we omitted the prefrontal cortex from our comparative analysis, even though it is considered part of the reward system in mammals, because its evolutionary antecedent in nonmammalian vertebrates is unclear (14). Furthermore, we combined distribution data for paralogous genes within a species (e.g., estrogen receptors ER α and ER β in vertebrates, teleost-specific androgen receptor duplicates AR α and AR β).

When comparing the distribution of neurochemicals across vertebrates, we observed several notable characteristics. First, the neurochemical profiles of the SDM network have been remarkably conserved throughout vertebrate

evolution (Fig. 2), consistent with their important roles in regulating behavior in all vertebrates. Secondly, an unsupervised clustering of neurochemical distributions across vertebrates segregated receptor distributions from ligands. When compared with receptors (Fig. 2 top), the spatial distribution of ligands (bottom) is significantly more restricted ($\chi^2 = 9.00$; $P = 0.011$). This analysis does not include aromatase, an enzyme that produces a ligand by converting testosterone to estrogen yet behaves like a receptor in terms of its wide distribution. Lastly, there appears to be more variation in where the ligands are produced (i.e., distribution of dopaminergic and neuropeptide-producing cells) than variation in where receptors are located, suggesting that the spatial distributions of neuroendocrine ligands are evolutionarily more flexible than those of receptors.

To explore the flexibility of neurochemical gene expression patterns across evolutionary time in a more quantitative manner, we calculated a divergence score (D) for each neurochemical gene product and brain region. We mapped distributions for each gene product across all SDM network nodes onto a vertebrate phylogeny and calculated D as the number of changes observed in the parsimonious model, where $D > 0$ indicates that one or more changes have occurred since tetrapods and ray-finned fishes shared their last common ancestor about 450 million years ago (Ma) (15). Figure 3A shows divergence scores for each brain region (columns) and neurochemical gene product (rows). The most-conserved ($D_{\text{avg}} = 0.0$) brain regions in our analysis are the basolateral amygdala (bAMY) and the preoptic area (POA), whereas the striatum (Str) is the least conserved ($D_{\text{avg}} = 0.7$). The average divergence scores for each brain region within either the reward system or social behavior network did not indicate that one system had undergone more changes than the other during vertebrate evolution (Fig. 3B; Mann-Whitney $U_{14} = 27.5$, $P = 0.662$). Furthermore, changes in neurochemical profiles in these two systems are uniformly distributed across the major transitions of vertebrate evolution (Fig. 3, C and D).

The neurochemical gene products whose distribution varies the most across the SDM network are TH ($D_{\text{avg}} = 0.6$), arginine vasopressin (AVP; $D_{\text{avg}} = 0.5$), and oxytocin (OXY; $D_{\text{avg}} = 0.4$), whereas the profiles of the oxytocin receptor (OTR; $D_{\text{avg}} = 0.0$) and the progesterone receptor (PR; $D_{\text{avg}} = 0.0$) are the most conserved. Profiles of both the androgen receptor (AR; $D_{\text{avg}} = 0.2$), and the dopamine D1 receptor (D1aR; $D_{\text{avg}} = 0.3$) exhibit little variation. To further examine potential differences in the spatial distribution of ligands and their receptors across vertebrate lineages, we averaged the divergence scores for each neurochemical gene product across brain regions and found that the variation in the spatial distribution of ligands (TH, aromatase, AVP, and OXY) is significantly greater than the variation in spatial distribution of receptors [AR, vasopressin

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1a receptor (V1aR), D1aR, ER, OTR, and PR; Fig. 3E] (Mann-Whitney $U_{10} = 2$, $P = 0.038$). Furthermore, the distribution of ligands exhibits a very heterogeneous temporal pattern with two dramatic changes (Fig. 3F). First, the number of SDM network nodes that contain TH-producing cells changed dramatically 450 Ma when the lineages that gave rise to teleosts (actinopterygians) and tetrapods (sarcopterygians) shared their last common ancestor, although without an

outgroup we cannot determine whether TH expression was lost or gained in these brain areas. Second, several SDM network nodes gained neuropeptide (AVP and OXY)-expressing cells when birds diverged from reptiles ~220 Ma or even longer ago. The information on reptiles in our analysis is largely limited to lepidosaurs (lizards and snakes), because data on turtles and crocodilians are sorely lacking. The latter group would be particularly interesting because of its

close relationship with birds in the archosaurian clade and because of the complex social behavior displayed by many of its representatives. Conversely, very few changes occurred in the spatial distribution of receptors over the past 450 million years (Fig. 3G). Aromatase could reasonably be grouped with either ligands or receptors, although this does not influence our results. To be conservative, we have grouped aromatase in the ligand class.

Because brain regions subserve different behaviorally relevant functions (5), we hypothesized that different SDM network nodes might have been subjected to varying selection pressures regarding their neurochemical profiles. We analyzed the neurochemical pattern of each brain region separately and found that brain regions show different degrees of neurochemical conservation across vertebrates (Fig. 4). We discuss here only the most-conserved and most-diverged brain regions (results for remaining regions are in fig. S1). The neurochemical profile of the pre-

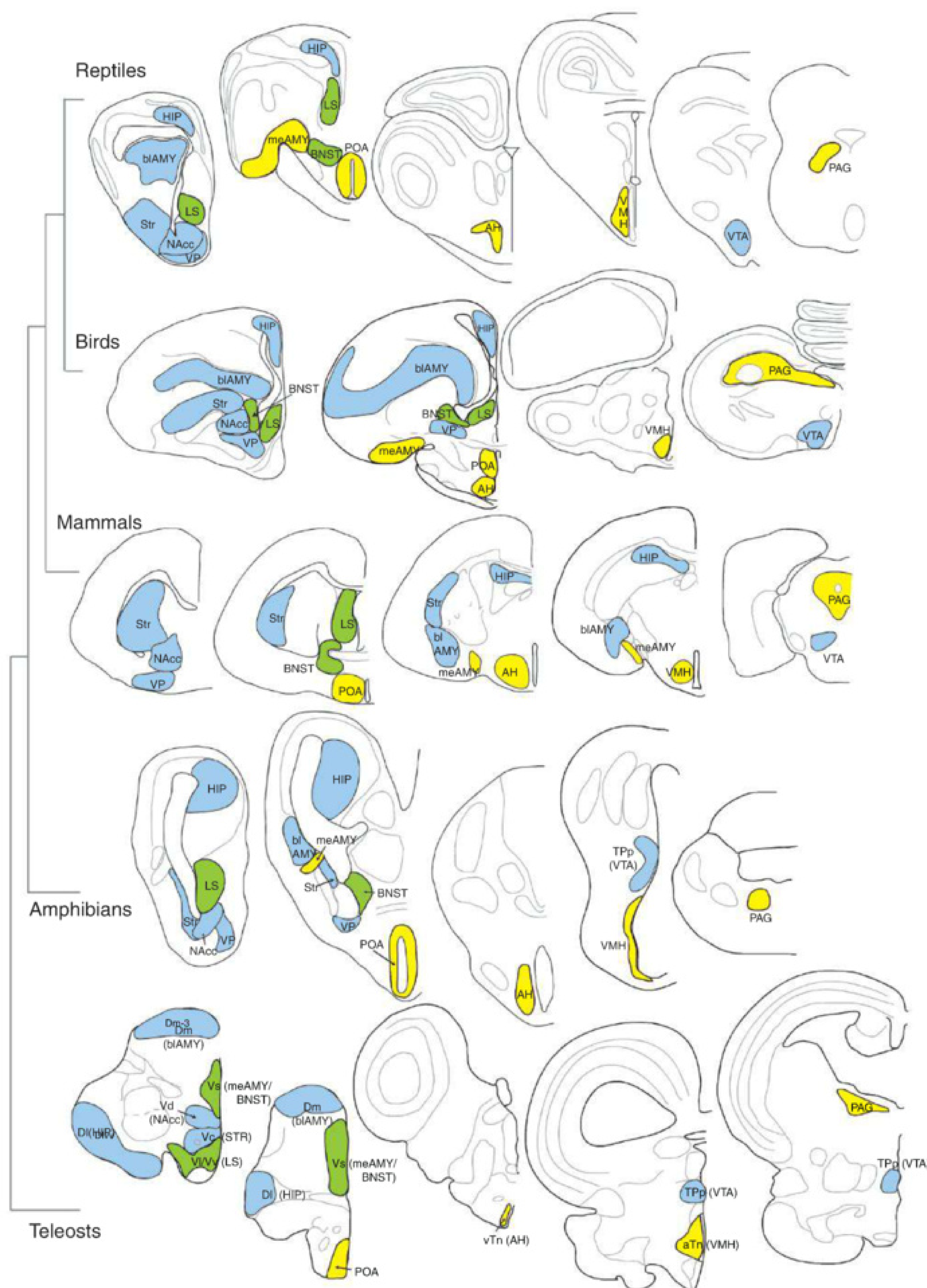


Fig. 1. Neural basis of behavioral diversity. The social behavior network (yellow) and mesolimbic reward system (blue) are two important neural networks regulating behavior in vertebrates and have functional connections (green) between the circuits. The mammalian nomenclature used in nonmammalian lineages does not necessarily imply discrete (one-to-one) homologs of mammalian structures. [Adapted from (5)] AH, anterior hypothalamus; BNST/meAMY, bed nucleus of the stria terminalis/medial amygdala; HIP, hippocampus; LS, lateral septum; NAcc, nucleus accumbens; PAG, periaqueductal gray/central gray; VMH, ventromedial hypothalamus; VP, ventral pallidum; VTA, ventral tegmental area.

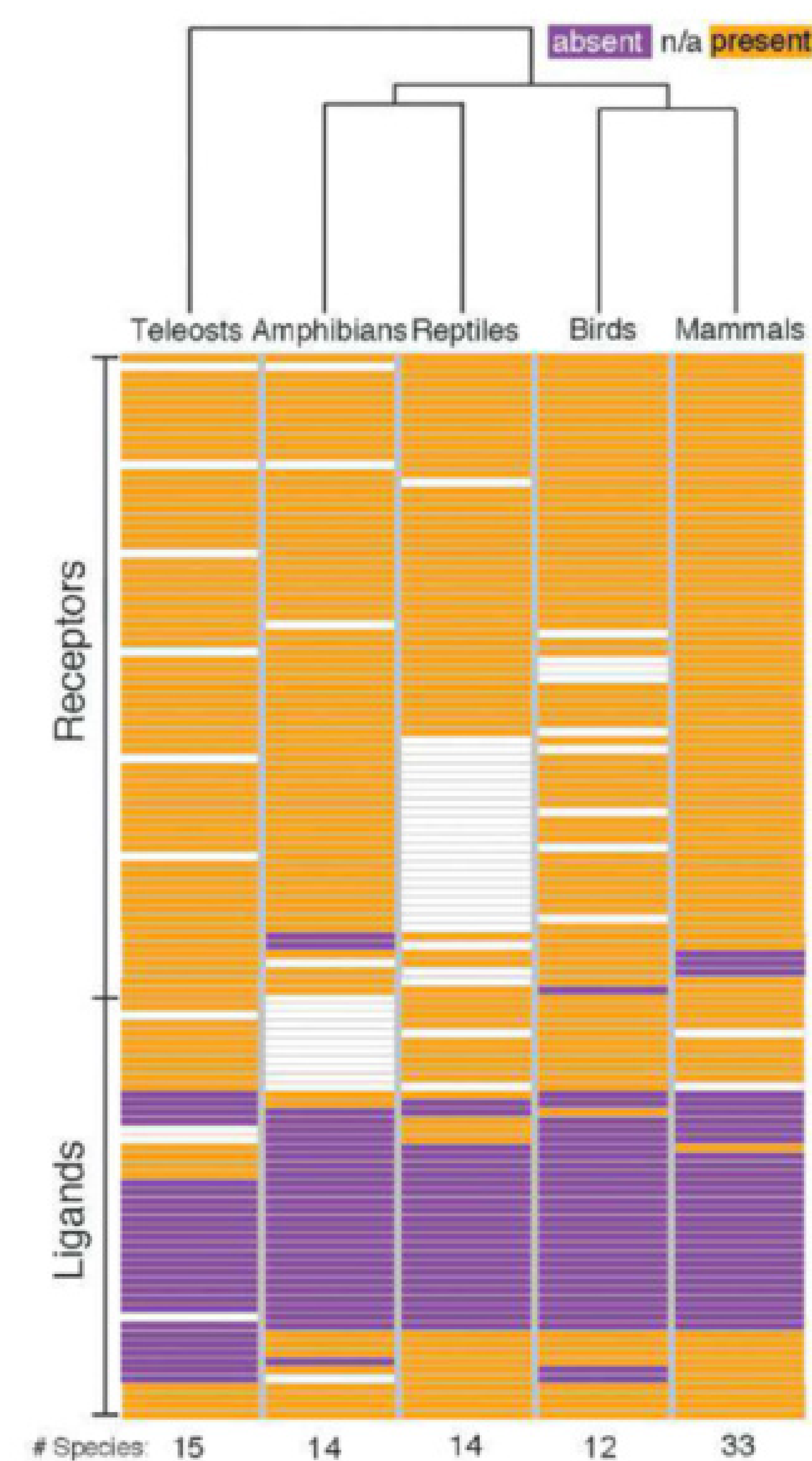


Fig. 2. Analysis of vertebrate patterns in brain neurochemistry. Overall patterns in neurochemistry in the social decision-making network are shown across vertebrates. Data for each vertebrate lineage are represented in the columns, and each row represents one gene in one brain region that is either present (orange) or absent (purple). White indicates that no data are available (n/a). The number of species in each vertebrate lineage represented in the analysis is shown at the bottom of each column. The dendrogram (top) is the result of unsupervised hierarchical clustering of the vertebrate neurochemical patterns.

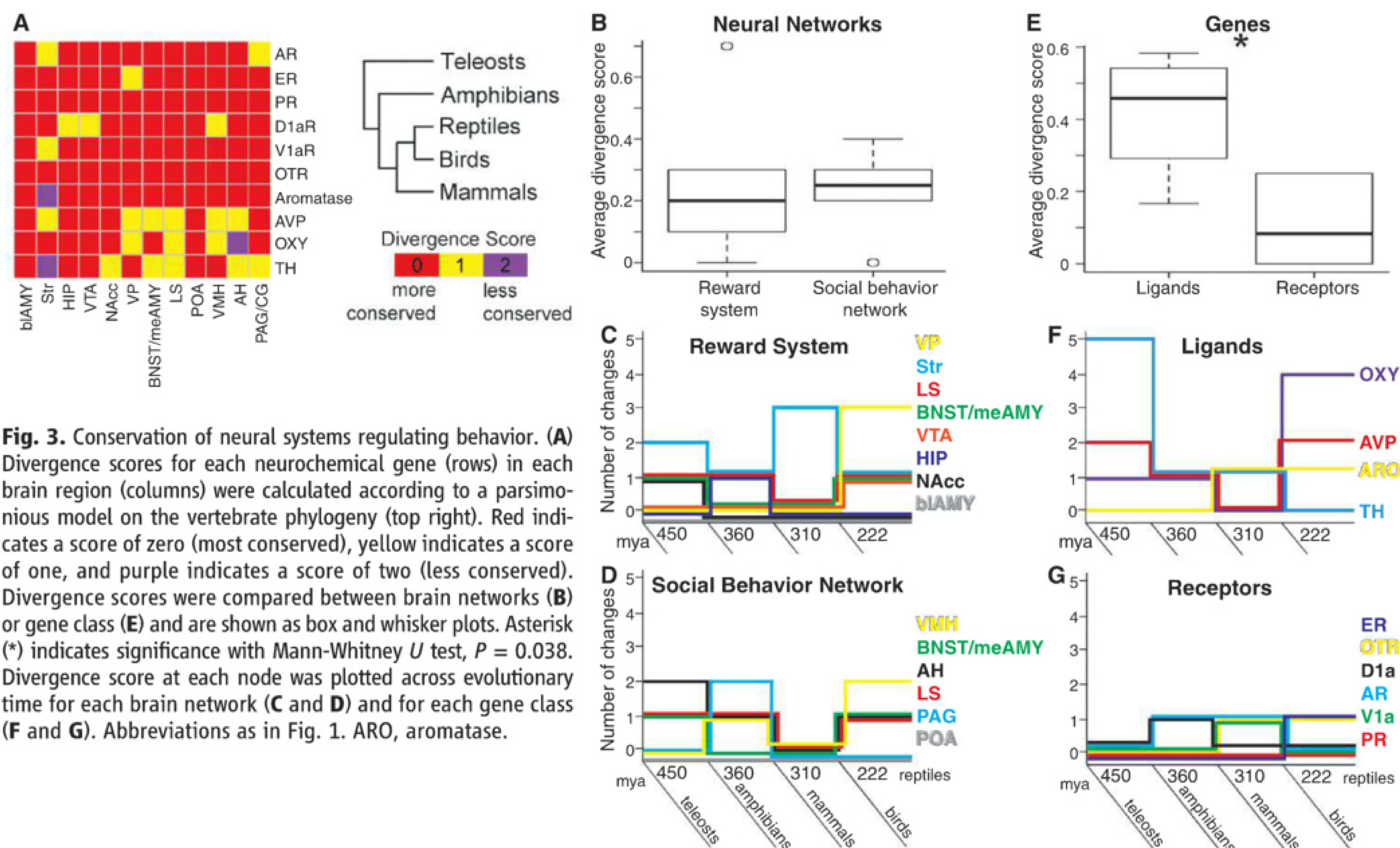


Fig. 3. Conservation of neural systems regulating behavior. **(A)** Divergence scores for each neurochemical gene (rows) in each brain region (columns) were calculated according to a parsimonious model on the vertebrate phylogeny (top right). Red indicates a score of zero (most conserved), yellow indicates a score of one, and purple indicates a score of two (less conserved). Divergence scores were compared between brain networks **(B)** or gene class **(E)** and are shown as box and whisker plots. Asterisk (*) indicates significance with Mann-Whitney *U* test, $P = 0.038$. Divergence score at each node was plotted across evolutionary time for each brain network **(C and D)** and for each gene class **(F and G)**. Abbreviations as in Fig. 1. ARO, aromatase.

optic area, a neuroendocrine relay station (16), is completely conserved, likely because of its fundamental role in coordinating and regulating basic behavioral and physiological functions. In contrast, neurochemical gene expression is most variable in the striatum, a multimodal receptive structure involved in reward processing and voluntary motor control (9). The extent to which the striatum receives pallial versus thalamic projections increased considerably at the anamniote-amniote and nonmammalian-mammalian transitions (17), a pattern that likely resulted in major functional shifts and resembles the changes in striatal gene profiles we have observed (Fig. 3C).

We next asked whether the variation seen across vertebrates might be confounded by species-level variation within a lineage. We examined the spatial patterns of dopamine-producing and AVP cells at the species level, because they are the best studied and most divergent across taxa. We found surprisingly little presence/absence variation within a vertebrate lineage in the distribution of dopaminergic or AVP-producing cells (fig. S2), suggesting that the patterns observed across vertebrates are not explained by variation within each lineage. However, within teleosts sites of dopamine production appear to show greater variation, possibly as the results of a more ancient origin and/or additional genome duplication events experienced by this group (18). Differences between closely related species that differ in social behavior have generally been reported as quantitative rather than qualitative (i.e.,

total presence or absence) with the exception of nonapeptide receptor expression in some mammalian and avian species (19, 20). Our analysis suggests that variation in neurochemical profiles within or across species from the same lineage is generally quantitative, as has been described within species with alternative phenotypes (21), between sexes (22), between species (23), and also in invertebrate behavioral systems (24), whereas large-scale patterns in gene expression variation across vertebrate lineages are generally qualitative (i.e., presence or absence).

We have examined the neurochemical profiles of brain regions important in social decision-making and found that, whereas the SDM network has overall been remarkably conserved over 450 million years of evolution, the sites of ligand production are evolutionarily more flexible than where their receptors are expressed, likely the consequence of two major expansions in ligand production sites ~450 and 222 Ma (25–27). The high conservation of receptor distributions may be explained by the ancient and conserved responses by animals to challenges and opportunities in their environment. Conversely, lineage differences in life history and ecology, coupled with phylogenetic constraints, might determine where and how social signals of different modalities are weighted and processed in the brain, possibly aided by differences in the local production of ligands. As a consequence, shifts in ligand expression to new sites in the brain resulting from small developmental changes (28)

might accompany the evolution of novel life history strategies or social systems, consistent with the known pleiotropic roles of many of these ligands. Recent investigations into the evolution of developmental mechanisms have similarly observed that the appearance of novel body plans or color patterns is often associated with changes in the spatial expression patterns of morphogenetically important ligands (29). On the other hand, receptors are generally associated with complex intracellular machinery and thus may be more restricted in spatial distribution changes than ligands, given that such a shift must also require altering the spatial distribution of many other proteins. Our analysis also suggests that macro- and microevolutionary processes might be dissociated, given that behavioral variation within a population or across closely related species can arise because of quantitative differences in receptor or ligand distributions (19, 24), although more comparative studies of these and other neurochemicals in nonmammalian taxa are needed to better understand how the social brain evolved with changes in brain gene expression.

Because the brain regions that govern social behavior function within a network, the integrative and systems-level approach we have used here opens up new avenues toward understanding the neural and molecular architecture of social behavior and its evolution. We suggest that the diversity of social behavior in vertebrates can be explained at least in part by variations on a conserved theme of neural and gene expression networks.

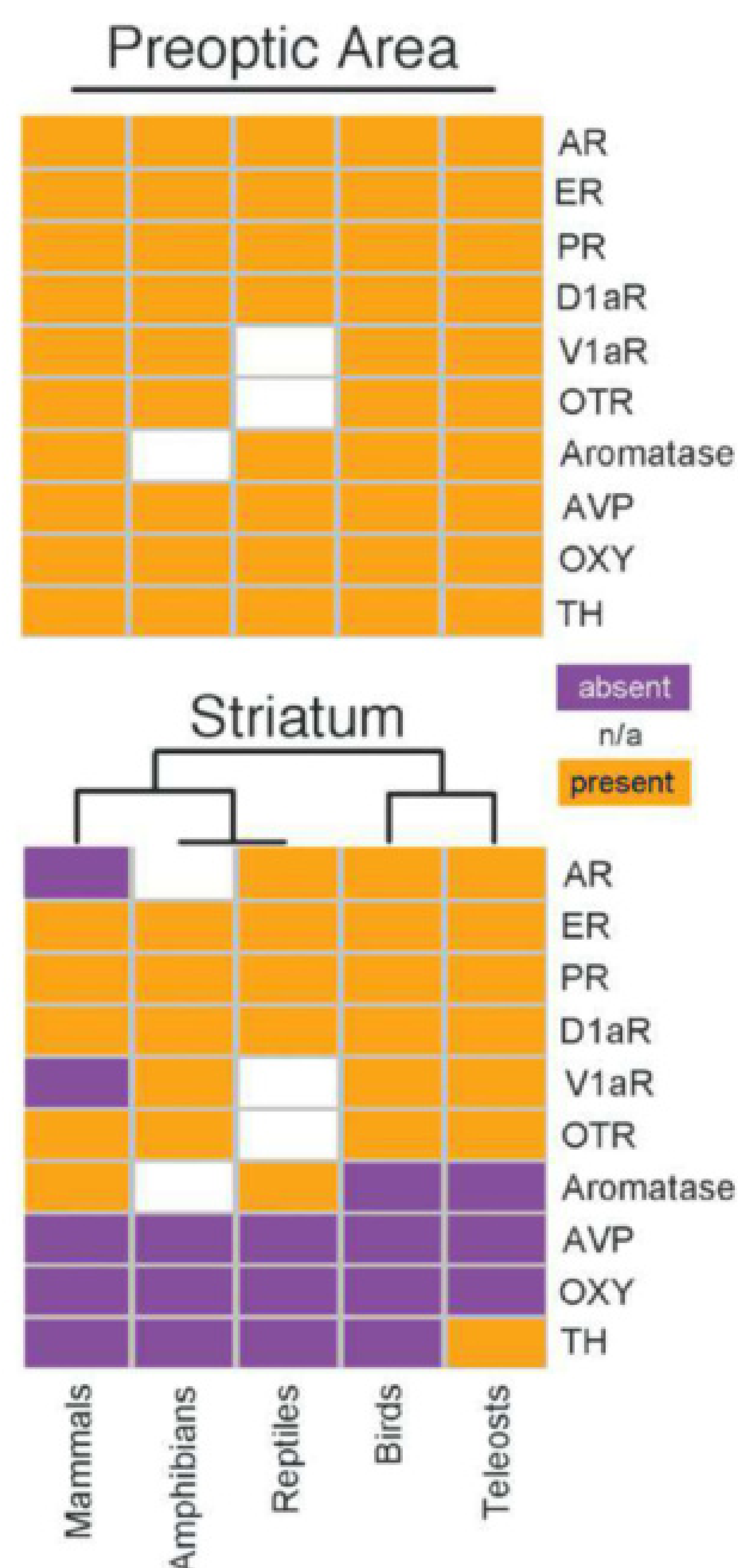


Fig. 4. The extent of conservation varies across brain regions. Patterns in neurochemistry are shown for the preoptic area (**top**) and striatum (**bottom**) where genes (rows) are either present (orange), absent (purple), or unknown (n/a, white) within each vertebrate lineage (columns). All other brain regions are shown in fig. S1.

Thus, our analysis provides a framework that will greatly facilitate the search for molecular universals underlying social behavior (30).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6085/1154/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S10
References (31–180)

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Evolutionary Trade-Offs, Pareto Optimality, and the Geometry of Phenotype Space

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Biological systems that perform multiple tasks face a fundamental trade-off: A given phenotype cannot be optimal at all tasks. Here we ask how trade-offs affect the range of phenotypes found in nature. Using the Pareto front concept from economics and engineering, we find that best-trade-off phenotypes are weighted averages of archetypes—phenotypes specialized for single tasks. For two tasks, phenotypes fall on the line connecting the two archetypes, which could explain linear trait correlations, allometric relationships, as well as bacterial gene-expression patterns. For three tasks, phenotypes fall within a triangle in phenotype space, whose vertices are the archetypes, as evident in morphological studies, including on Darwin's finches. Tasks can be inferred from measured phenotypes based on the behavior of organisms nearest the archetypes.

Consider a biological system whose phenotype is defined by a vector of traits, v . Traits considered here are quantitative measures such as bird beak length and not genetic traits such as DNA sequences. The space of all phenotypes is called the morphospace. Most theories of natural selection maximize a specific fitness function $F(v)$, resulting in an optimal phenotype, usually a point in morphospace. This approach has several limitations: First, the fitness function is often unknown. Second, in many cases, organisms need to perform multiple tasks that all contribute to fitness (I); thus, fitness is

an increasing function of the performance at all tasks $F(P_1(v), \dots, P_k(v))$, where $P_i(v)$ is the performance at task i . The best phenotype for one task is usually not the best for other tasks—resulting in a trade-off situation. Maximizing fitness is thus a multi-objective optimization problem (2–5).

To address this issue, we employ the Pareto front concept (2–6), used in engineering and economics to find the set of designs that are the best trade-offs between different requirements. Consider two phenotypes v and v' . If v' is better at all tasks than v , the latter will be eliminated by natural selection (Fig. 1A). Repeating this for all possible phenotypes, one remains with the Pareto front: the set of phenotypes that cannot be improved at all tasks at once. The Pareto front describes all optima for all conceivable fitness functions that are increasing functions of the

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performance in each task. Which of the phenotypes on the front is selected depends on the relative contributions of each task to the organism's fitness in its natural habitat, provided that evolution has had sufficient time and genetic variance to reach the predicted point.

The Pareto front is typically a small region of morphospace. This may explain the long-standing observation that most of morphospace is empty (7): Phenotypes such as animal shapes found in nature fill only a small fraction of morphospace.

We next calculate the Pareto front in morphospace. This requires two assumptions (which will be relaxed below). (i) Each performance function is maximized by a single phenotype. The phenotype that is best at task i will be called the archetype for task i , denoted v_i^* . (ii) Performance decreases with distance from the archetype (Fig. 1B). By distance, we mean a metric based on an inner product norm, such as Euclidean distance [mathematically, $P_i(v) = P_i(d_i)$, where $d_i = (v - v_i^*)^T M (v - v_i^*)$, and M is a positive-definite matrix; Euclidean distance $d_i = (v - v_i^*)^2$ is when $M = I$]. For two tasks, geometric considerations show that the Pareto front is the line segment that connects the two archetypes (Fig. 1B). This is because any point off the line segment is farther from both archetypes than its projection on the line—thus points off the line have lower performance at both tasks, and hence lower fitness, and will be selected against. The position of a phenotype on the line relates to the relative importance of the two tasks in the habitat in which the organism evolved: the closer to an archetype, the more important that task (8).

The case of a trade-off between two tasks may explain the widespread occurrence of linear relations between traits (2, 9, 10). As an example, the area proportions of the molar teeth of 29 rodent species show an approximately linear relationship (11) (Fig. 1C). Species are distributed along the line according to their diet: herbivores at one end, carnivores at the other, and omnivores in the middle. Thus, the archetypes correspond to the ends of the observed line segment: a herbivore archetype with equal-sized molars, and a carnivore archetype with molars in the ratio 2:1:0. Omnivore molars are weighted averages of these archetypes. As in many morphological studies, the traits here are normalized to account for organism size: Because all molar areas scale with size, taking the ratio of molars removes the effect of organism size variation (8). Additionally, the present theory might explain cases of allometry, when traits depend on total organism size (9–12). Allometric relations often behave as power laws, observed as lines in logarithmic plots—predicted when the performance decays with a metric that is a function of the log of the traits (8), as suggested, for example, by scaling laws for metabolic transport (12). Other explanations for allometric relations include physical or developmental constraints (10, 11).

For more than two tasks, the Pareto front is the full polygon or polyhedron whose vertices are

the archetypes (8) (Fig. 2) [or, equivalently, the convex hull of the archetypes, defined as the set of all points that are weighted averages of the archetypes: $v = \sum_{i=1}^k \theta_i v_i^*$ with nonnegative weights θ_i that sum to one. For particular fitness and performance functions, the weights can be calculated:

$$\theta_i = \frac{\partial F / \partial P_i}{\partial F / \partial P_i} / \sum_{j=1}^k \frac{\partial F / \partial P_j}{\partial F / \partial P_j}.$$

Weights sum to one $\sum_{i=1}^k \theta_i = 1$, and they are nonnegative, $\theta_i \geq 0$, because fitness increases with performance $\partial F / \partial P_i \geq 0$ and performance decreases with distance from its archetype $\partial P_i / \partial d_i < 0$ (8)]. For three tasks, the Pareto front is the full triangle whose vertices are the three archetypes. In this case, because a triangle defines a plane, even high-dimensional data on many traits are expected to collapse onto two dimensions. The closer a point is to one of the vertices of the triangle, the more important the corresponding task is to fitness in the organism's habitat.

We find evidence for such triangular suites of variation in several classic studies of animal morphology and evolution. In these studies, there was no theory to explain why the data resemble a

triangle. The species near the vertices of the triangles have distinct behavior that suggests which task is optimized by each archetype (Fig. 3, A to C). A triangle is found in the study of Grant and colleagues on Darwin's finches (13) (Fig. 3A). Measurements of five beak and body traits (five-dimensional morphospace) fall on a two-dimensional plane: Two principal components—related to body size and beak shape—account for 99% of the variation (8). On this plane, the data fall within a triangle [$P < 10^{-4}$, according to a statistical test of triangularity; Fig. 3A, inset (8, 14)]. The triangle suggests three archetypes, one at each vertex. The species near the archetypes suggest which tasks may be optimized by each archetype, in this case tasks connected with diet: (1) probing for insects and nectar (long beak, cactus finch), (2) crushing large, hard seeds (thick beak, large ground finch), and (3) crushing small, soft seeds (small beak, small ground finch). Intermediate finch species perform a combination of these tasks (8).

We also noted a triangle-shaped suite of variation in E. O. Wilson's study of leaf-cutter ants (15) [Fig. 3B, $P < 10^{-4}$ (8, 14)]. The three

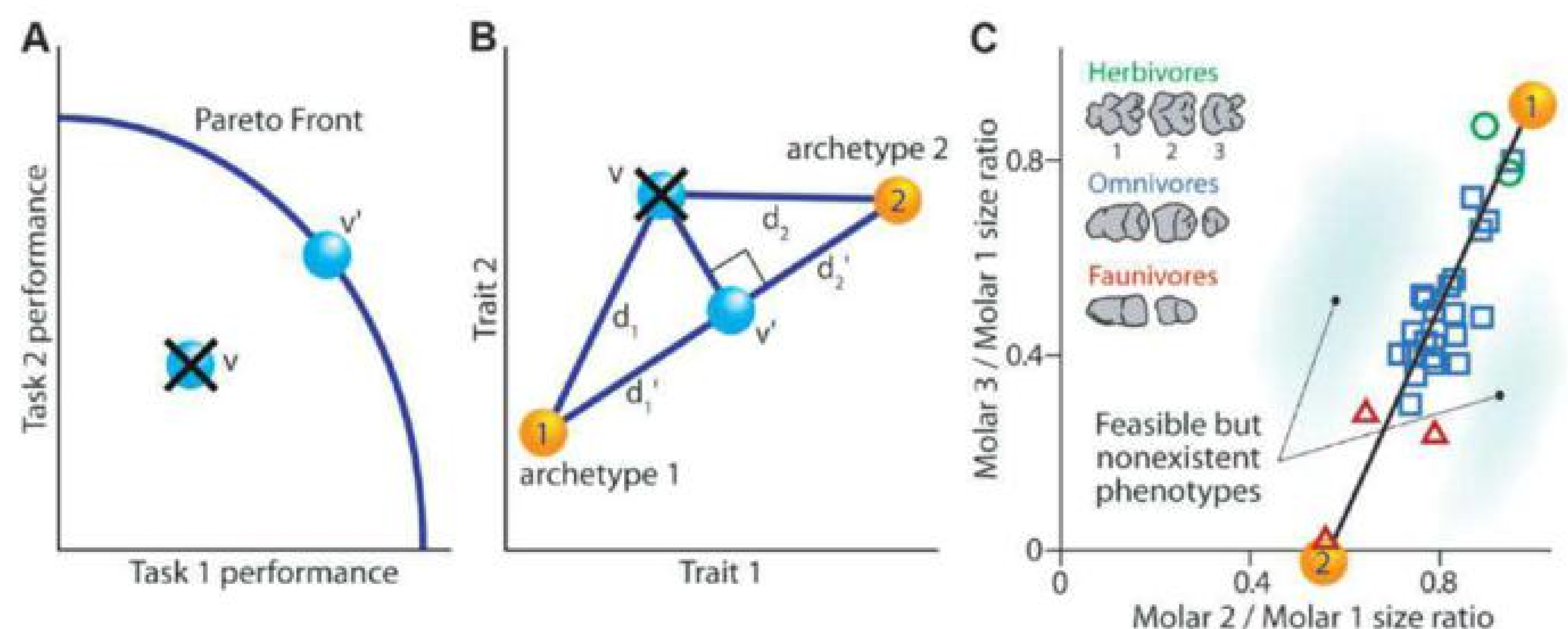


Fig. 1. (A) The Pareto front (best trade-offs) is what remains after eliminating (crossed-out symbol) all feasible phenotypes v that are dominated on all tasks by other feasible phenotypes v' . (B) The two archetypes in morphospace maximize performance in tasks 1 and 2. Phenotype v is farther from both archetypes than v' , its projection on the line segment that connects the archetypes. Thus, v has lower performance than v' in both tasks, hence lower fitness. Eliminating all such points v , one remains with the Pareto front: the line segment connecting the two archetypes [unlike (A), axes are traits, not performances]. (C) The area ratios of rodent molar areas show a linear relationship (11). Most of the morphospace is empty. Herbivores (circles), faunivores (triangles), and omnivores (squares) are indicated.

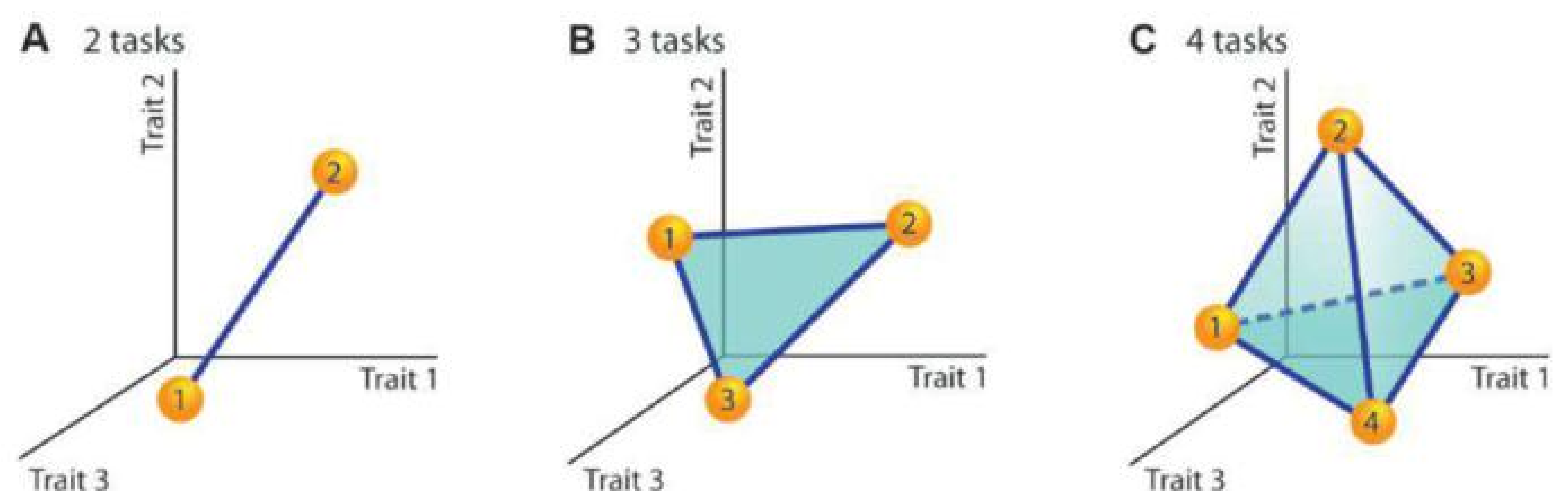


Fig. 2. Pareto front geometry. (A) Two tasks form a line (B) Three tasks form a triangle. (C) Four tasks form a tetrahedron. If only some relevant traits are measured and others are not, lines and triangles should still be found, because a projection of a convex hull on a subspace is still a convex hull (8). The distribution of phenotypes along the front depends on the second derivative of the performance and fitness functions (8).

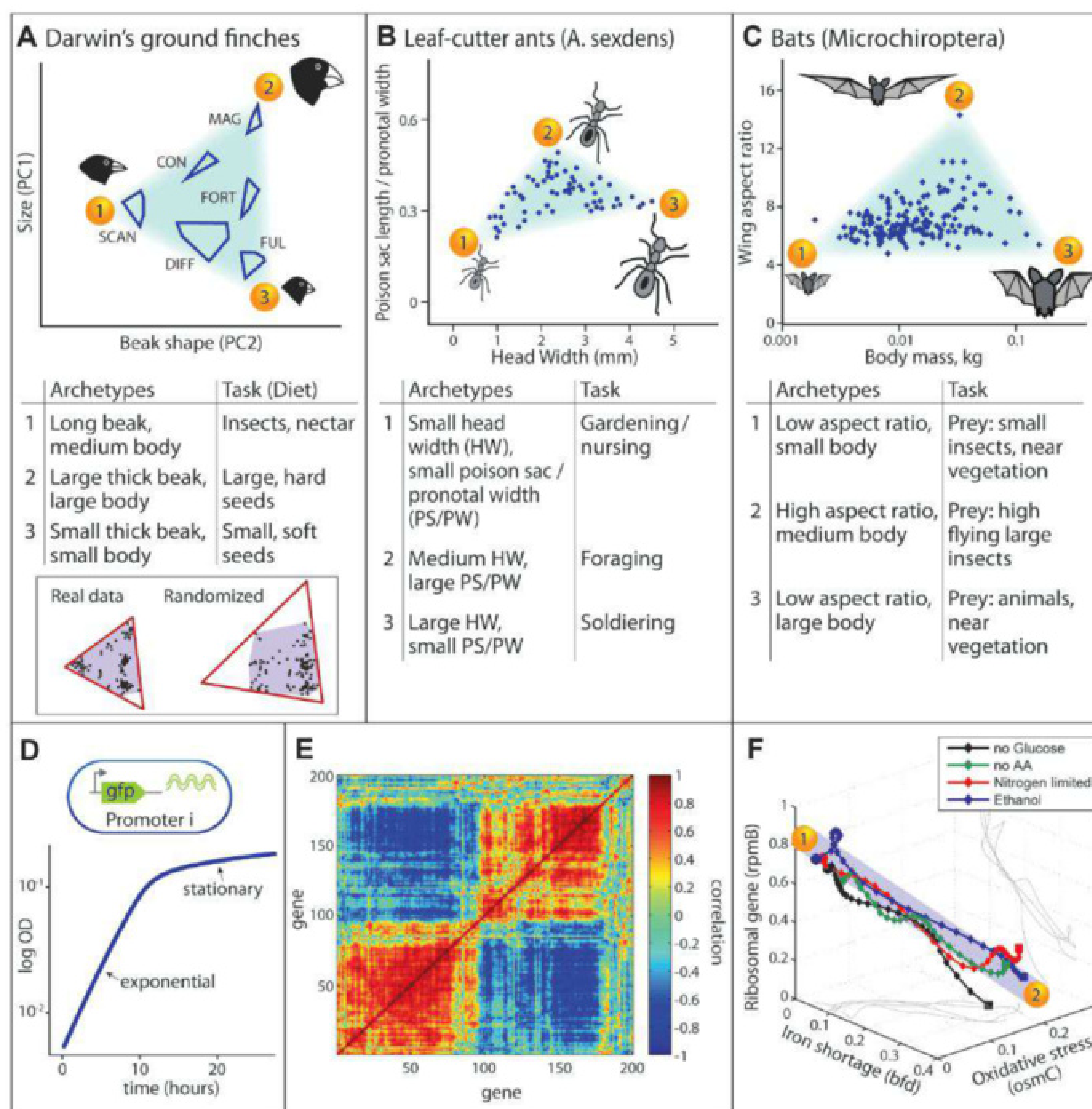
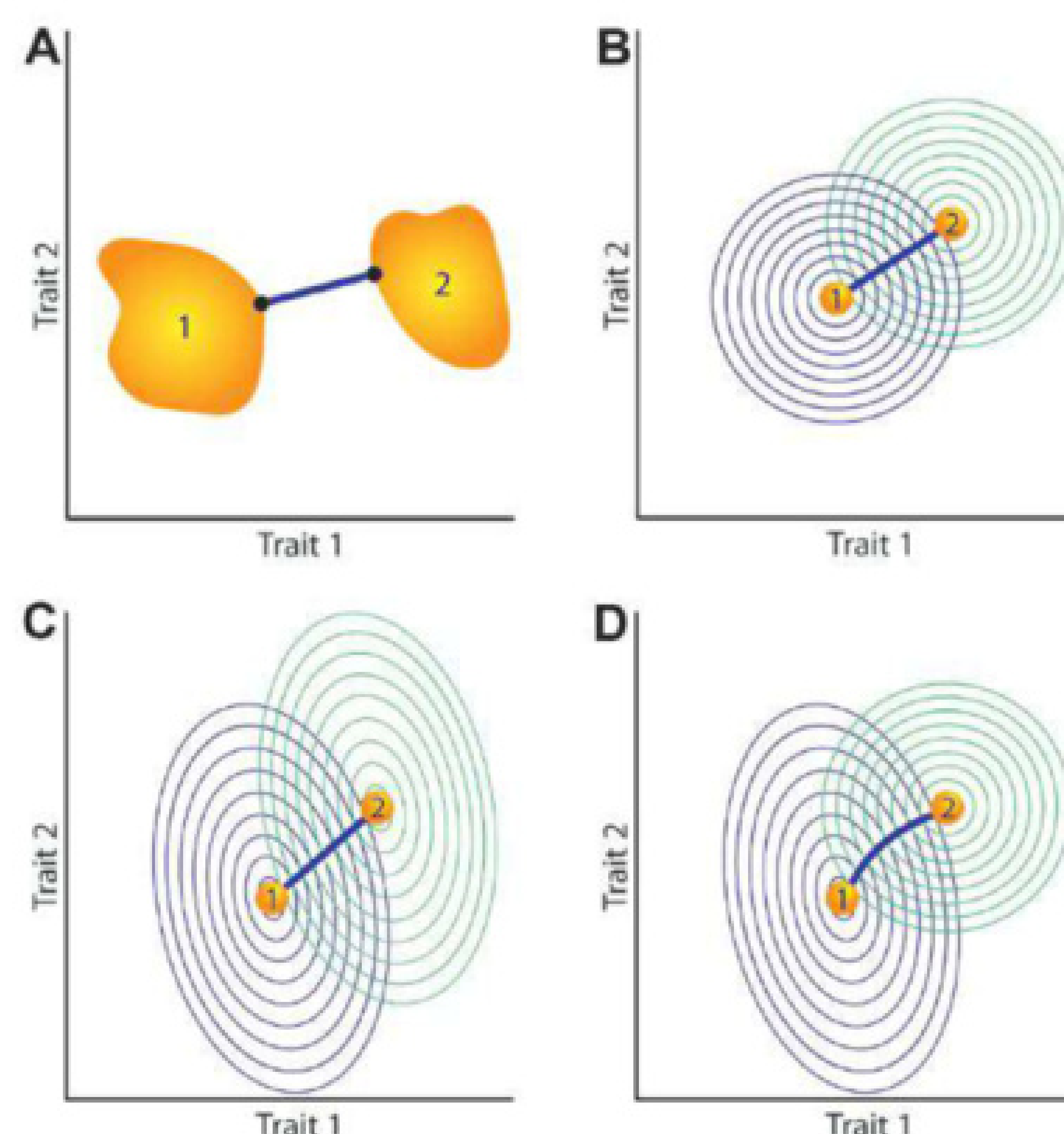


Fig. 3. Triangular suites of variation, and trade-offs in *E. coli* gene expression. **(A)** Darwin's ground finches (13). Axes correspond to size and beak shape. Polygons are boundaries of intraspecies variation. See (8) for species definitions. Inset: Statistical test for triangularity. Define t ratio as the ratio of the area of the minimal-area triangle (red) to the area of the convex hull of the data (purple). The P -value is the fraction of times that randomized data have a larger t ratio than the real data, based on 10^4 randomized data sets that preserve the statistics of each trait independently (8). **(B)** Leaf-cutter ant (*Atta sexdens*) (15): poison sac (pheromone gland that marks the trail) length (normalized to pronotal width) versus head width. **(C)** Bat (Microchiroptera) wing aspect ratio versus body mass (16). Archetypes and inferred tasks are listed below each figure. **(D)** *E. coli* promoter activity was measured with fluorescent reporters (18). **(E)** Clustered correlation matrix of the top 200 temporally varying genes reveals two anticorrelated clusters. **(F)** Percentage of total promoter activity of three genes at different time points, in four different media conditions (8), as bacteria transit from exponential phase (1) to stationary phase (2).

Fig. 4. **(A)** Relaxing assumption (i): When performance is maximized in a region rather than a single point, the Pareto front is the line that connects the closest point in the region to the other archetypes (8). **(B and C)** When all performance functions decay with the same distance metric, the Pareto front is a straight line. The front is the set of tangent points between equi-performance contours (8). **(D)** Relaxing assumption (ii): When each performance function decays with a different metric (different elliptical contours), the front is slightly curved. Root mean square deviation from a straight line is 21%, averaged over ellipses of all orientations and major/minor axis ratios spanning a hundredfold range (8). For three tasks, triangles with curved edges are generally found.



archetypes are associated with nursing/gardening, foraging outside the nest, and soldiering. Intermediate ants perform a combination of these tasks. Additionally, Norberg and Rayner's study of bat wings (16) [Fig. 3C, $P < 3 \times 10^{-2}$ (8, 14)] shows a triangular pattern. Archetypes seem to be associated with eating insects in vegetation, hawking insects in the air far above vegetation, and eating large prey in vegetation.

The present considerations might apply beyond animal morphology. For example, bacteria face a trade-off in partitioning the total amount of proteins they can make at a given moment between the different types of proteins—that is, how much of each gene to express. A given expression pattern cannot be optimal, at the same time, for two different tasks such as rapid growth (which requires ribosomes) and survival (which requires stress response proteins) (17). Thus, the theory predicts that gene-expression patterns fall on low-dimensional Pareto fronts, whose vertices are archetypal expression patterns optimal for a single task.

We tested this hypothesis on *Escherichia coli* gene expression (Fig. 3D). The activity of 1600 promoters was tracked with fluorescent reporters as bacteria grew from exponential to stationary phase (18). Activity was normalized by the summed activity of all promoters at each time point, to represent the instantaneous allocation of transcription resources. The top 200 temporally varying promoters account for 96% of the total temporal variation and control genes in two main families (Fig. 3E) (8): growth genes (ribosomes, transcription, and translation) and stress/survival genes (oxidative stress, etc.). This high-dimensional data set falls on a line [Fig. 3F, $P < 10^{-4}$ (8), fig. S10]. At one end, expression is devoted mostly to growth genes (exponential phase, archetype 1), and at the other end expression is devoted primarily to stress/survival genes (stationary phase, archetype 2). Over time, the expression program gradually moves along the line from archetype 1 to 2. The instantaneous allocation at each time point is, to a good approximation, a weighted average of two archetypal expression programs: growth and survival. Similar analysis may explain low-dimensional patterns in gene-expression measurements in bacteria (19) and cancer cells (20).

Relaxing the assumptions (i) and (ii) above generally preserves the topology of the Pareto front, with mildly curved lines instead of straight edges, but nevertheless with distinct vertices that can be related to archetypes (Fig. 4) (8).

The present theory addresses traits that have a trade-off. If a trade-off does not exist, trait values can vary independently. Observed phenotypes in this case may fill an uncorrelated cloud in morphospace (8).

Variation in traits within a population in a given species often falls on the same line as variations between species—a phenomenon called “evolution along genetic lines of least resistance” (21). This can be explained by the present frame-

work: Variation within a species reflects the range of habitats it inhabits, each with differential importance of the tasks. Thus, populations should be distributed on the same Pareto front as different species facing the same tasks.

Finally, Pareto optimality need not be the only or generic explanation for low dimensionality and lines/triangles in biological data. It may work for some examples and not others, especially if biological constraints other than natural selection are important. The following experimental tests can refute the theory in a specific example: (i) A point in the middle of the front has higher performance in one of the tasks than a point close to the relevant vertex (this might also imply that different tasks are at play). (ii) A mutant can be found that has higher performance at all tasks than existing phenotypes. Both of these tests require measuring performance (*I*, *7*, *13*)—but not the more difficult task of measuring fitness. (iii) Laboratory evolution experiments can follow a mutant that is off the Pareto front (has lower performance in all tasks than the wild type), under conditions in which all tasks are required. Provided with sufficient genetic variation, the

mutant is predicted to evolve phenotypes closer to the front.

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Supplementary Materials

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Chitin-Induced Dimerization Activates a Plant Immune Receptor

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Pattern recognition receptors confer plant resistance to pathogen infection by recognizing the conserved pathogen-associated molecular patterns. The cell surface receptor chitin elicitor receptor kinase 1 of *Arabidopsis* (AtCERK1) directly binds chitin through its lysine motif (LysM)—containing ectodomain (AtCERK1-ECD) to activate immune responses. The crystal structure that we solved of an AtCERK1-ECD complexed with a chitin pentamer reveals that their interaction is primarily mediated by a LysM and three chitin residues. By acting as a bivalent ligand, a chitin octamer induces AtCERK1-ECD dimerization that is inhibited by shorter chitin oligomers. A mutation attenuating chitin-induced AtCERK1-ECD dimerization or formation of nonproductive AtCERK1 dimer by overexpression of AtCERK1-ECD compromises AtCERK1-mediated signaling in plant cells. Together, our data support the notion that chitin-induced AtCERK1 dimerization is critical for its activation.

In plants, pathogen-associated molecular pattern (PAMP)-induced immunity is mediated by the typically membrane-anchored proteins (*1–3*) pattern recognition receptors (PRRs), most of which are receptor-like kinases (RLKs) (*4*). Several PRRs (*5–9*) have been identified, including those critical for chitin-induced immune responses. Chitin, a polymer of *N*-acetyl-D-glucosamine (NAG), is a well-known PAMP that elicits plant immunity (*10*). The first chitin receptor identified in rice (*Oryza sativa*), OsCEBiP (*9*), carries an extracellular lysine motif (LysM) domain that is widely distributed for NAG recognition (*11*). In *Arabidopsis*, a CEBiP homolog, AtCERK1 (*5*) or LysM RLK1 (*6*), is required for chitin-triggered immunity. LysM-containing receptors appear to have a conserved role in

chitin perception, as they also contributed to chitin-induced plant defenses in other species (*12, 13*). AtCERK1 is also involved in detecting the bacteria-derived peptidoglycans (PGNs) to mediate *Arabidopsis* immunity (*14, 15*). Besides plant defenses, LysM-containing proteins recognize the chitin-related molecules, Nod factors, to initiate root nodulation (*16*).

AtCERK1 has been established as a chitin receptor (*5, 6, 17, 18*), and the AtCERK1-ECD containing three tandem LysMs (LysM1–3) directly recognizes chitin to signal plants for immunity (*17, 18*). Chitin binding induces phosphorylation of the intracellular kinase domain of AtCERK1 (*17*) and activates disease resistance (*5, 6, 10, 19, 20*). Here we present biochemical, molecular, and structural data (table S1) supporting a model in

which chitin-induced oligomerization is important for AtCERK1 activation, providing a template for understanding PAMP-induced PRR activation.

The three LysMs pack tightly against each other, resulting in a globular structure. Each LysM contains a $\beta\alpha\alpha\beta$ structure in which the two β strands form an antiparallel β sheet (Fig. 1). The three LysMs share a conserved architecture (fig. S1A) that is similar to those of other LysM-containing proteins (fig. S2). The comparatively conserved residues among the three LysMs are limited to the $\beta\alpha\alpha\beta$ regions (fig. S1B). Though making few contacts with each other, LysM2 and LysM3 pack tightly against LysM1. β 1 in LysM1 and its counterpart β 5 in LysM3 form a parallel β sheet, relating the two LysMs in a quasi two-symmetry axis (Fig. 1, left). LysM1 and LysM2 are also related by a quasi two-symmetry axis but through packing of different structural elements (Fig. 1, right). The tight packing of the three LysMs suggests that deletion of one LysM could

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generate a deleterious effect on the structural integrity of the other two.

The CXC motif harbored in the variable intervening sequences between the LysMs of AtCERK1 is conserved among the LysM-containing RLKs (5, 6, 9, 16). Together with the N-terminal two cysteine (Cys) residues of LysM1, the four Cys residues from the two CXC motifs of AtCERK1 make three pairs of disulfide bridges (fig. S1B).

Five potential glycosylation sites (fig. S3) defined by sufficient electron density (not shown) were found in the AtCERK1-ECD.

Despite the similarity of the three LysMs, only LysM2 was found to bind to (NAG)₅. However, it remains undetermined whether the other two LysMs are capable of binding to chitin. No conformational change occurs to AtCERK1-ECD after chitin binding (fig. S4). Electron density de-

fines four of the five NAGs anchoring to a shallow groove that is created by two loops (L1 and L2) and lined with a few cavities at the bottom (Fig. 2A). A similar chitin-binding region was also found in another LysM-containing protein (21). The NAG units exhibit an alternation of ~180° flipping along the chain, adopting a fully extended conformation closely matching the surface topology of the groove (Fig. 2A).

The rings of NAG-2 and NAG-3 stack against L2, whereas no interaction exists between the remaining two rings and LysM2 (Fig. 2B). (NAG)₅-LysM2 interactions are established mainly through some of the branched groups from one side of (NAG)₅, providing numerous hydrogen bonds with the main chain of AtCERK1-ECD (Fig. 2B). Specific recognition of the *N*-acetyl moieties in NAGs allows AtCERK1 to distinguish chitin from glucose. In NAG-4, the carbonyl oxygen of the *N*-acetyl portion hydrogen bonds with the backbone-nitrogen of Glu¹¹⁴, whereas the methyl group engages contacts with the side chains of Met¹²⁷, Gln¹³¹, and Ala¹³⁸. A water molecule fills between NAG-4 and LysM2, bridging a hydrogen bond between the C3 hydroxyl group and the main-chain nitrogen of Tyr¹¹³. NAG-2 inserts its *N*-acetyl group deeply into a clear-cut pocket, allowing its carbonyl oxygen and amide nitrogen to hydrogen bond with the main-chain nitrogen

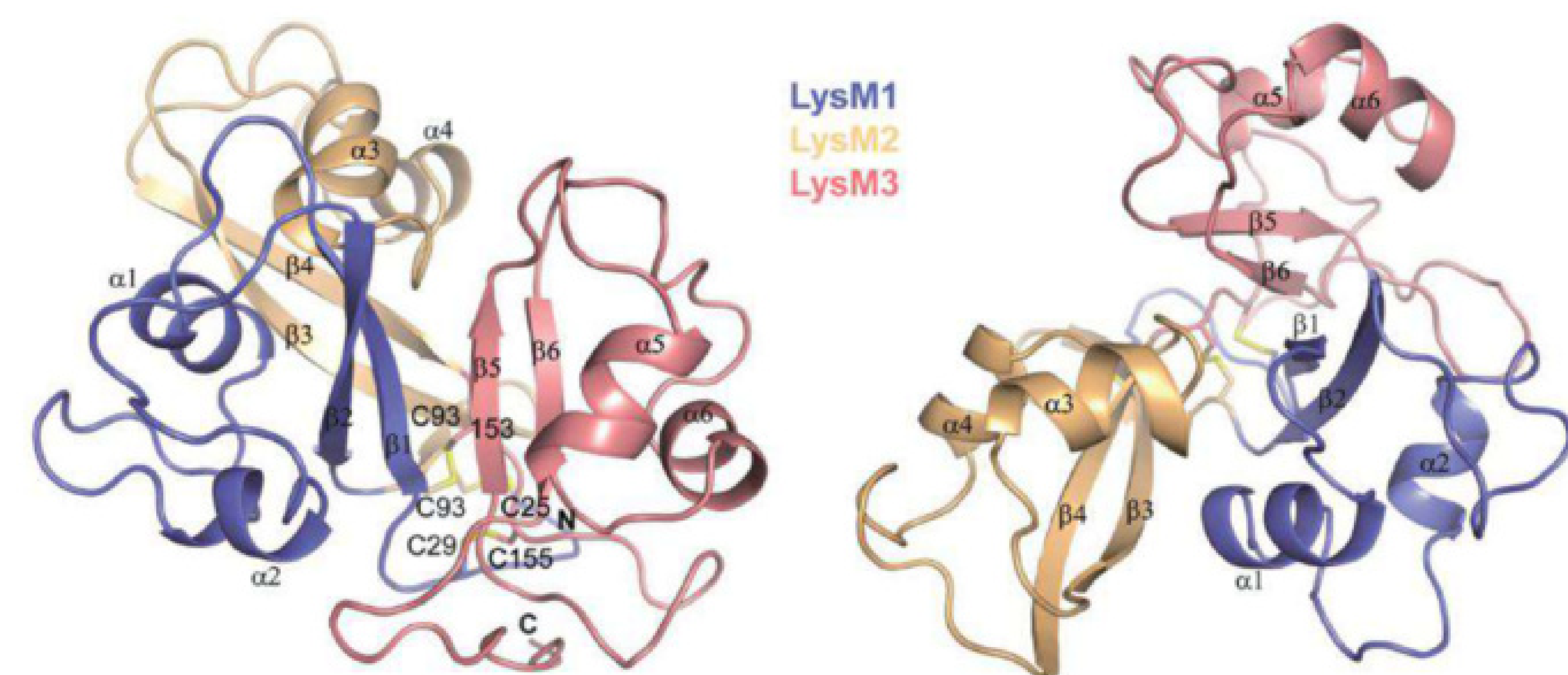


Fig. 1. Tight packing of the three LysMs in AtCERK1-ECD. Overall structures of AtCERK1-ECD in two different orientations. The secondary-structure elements are labeled. Disulfide bonds (C29-C155, C25-C93, and C93-C153) are labeled and shown in yellow stick.

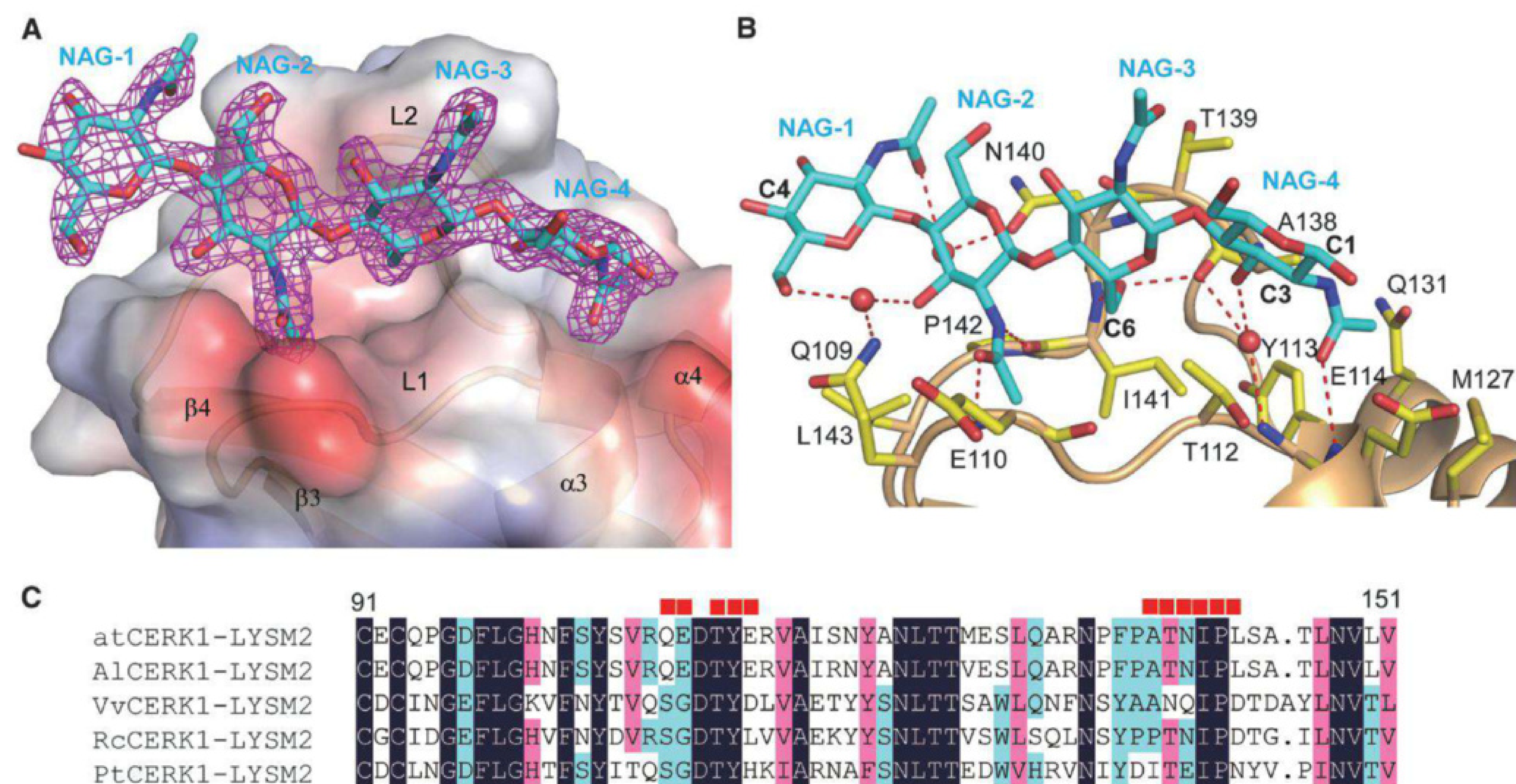


Fig. 2. Specific recognition of a chitin oligomer by AtCERK1-ECD. (A) Chitin binds to a shallow surface groove on AtCERK1-ECD. AtCERK1-ECD is shown in electrostatic surface (transparency) and cartoon. White, blue, and red indicate neutral, positive, and negative surfaces, respectively. Shown in magenta mesh is the omit electron density ($F_o - F_c$, 2.8σ) around the bound chitin oligomer. Chitin residues are labeled (NAG1-4). (B) Detailed interactions between chitin and the AtCERK1-ECD. The side chains from AtCERK1 are shown in yellow. Red spheres represent oxygen atoms of water molecules. Four carbon atoms of the

chitin oligomer are indicated (C1, C3, C4, and C6 in bold). The distance cut-off is 3.4 Å. (C) Sequence alignment of CERK1-LysM2 from different species. Red squares indicate the residues from LysM2 involved in interaction with chitin. At: *Arabidopsis thaliana*; Al: *Arabidopsis lyrata*; Vv: *Vitis vinifera*; Rc: *Ricinus communis*; Pt: *Populus trichocarpa*. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

of Glu¹¹⁰ and the carbonyl oxygen of Ile¹⁴¹, respectively (Fig. 2, A and B). NAG-3 is stabilized by van der Waals interactions between the hydroxymethyl group (C6) and its surrounding residues. Additionally, the C6 hydroxyl of NAG-3 participates in cooperative hydrogen bonds involving the backbone nitrogen of Ile¹⁴¹ and carbonyl oxygen of Ala¹³⁸ (Fig. 2B). Interaction of NAG-1 with LysM2 is exclusively through water-mediated hydrogen bonds. The residues around the chitin-binding site of LysM2 are highly variable in the other two LysMs (fig. S1B) but comparatively conserved among different plants (Fig. 2C).

As both ends of the bound chitin fragment protrude out into the solvent region, extension of the chitin chain to either direction would not provide many additional, if any, chitin-LysM2 inter-

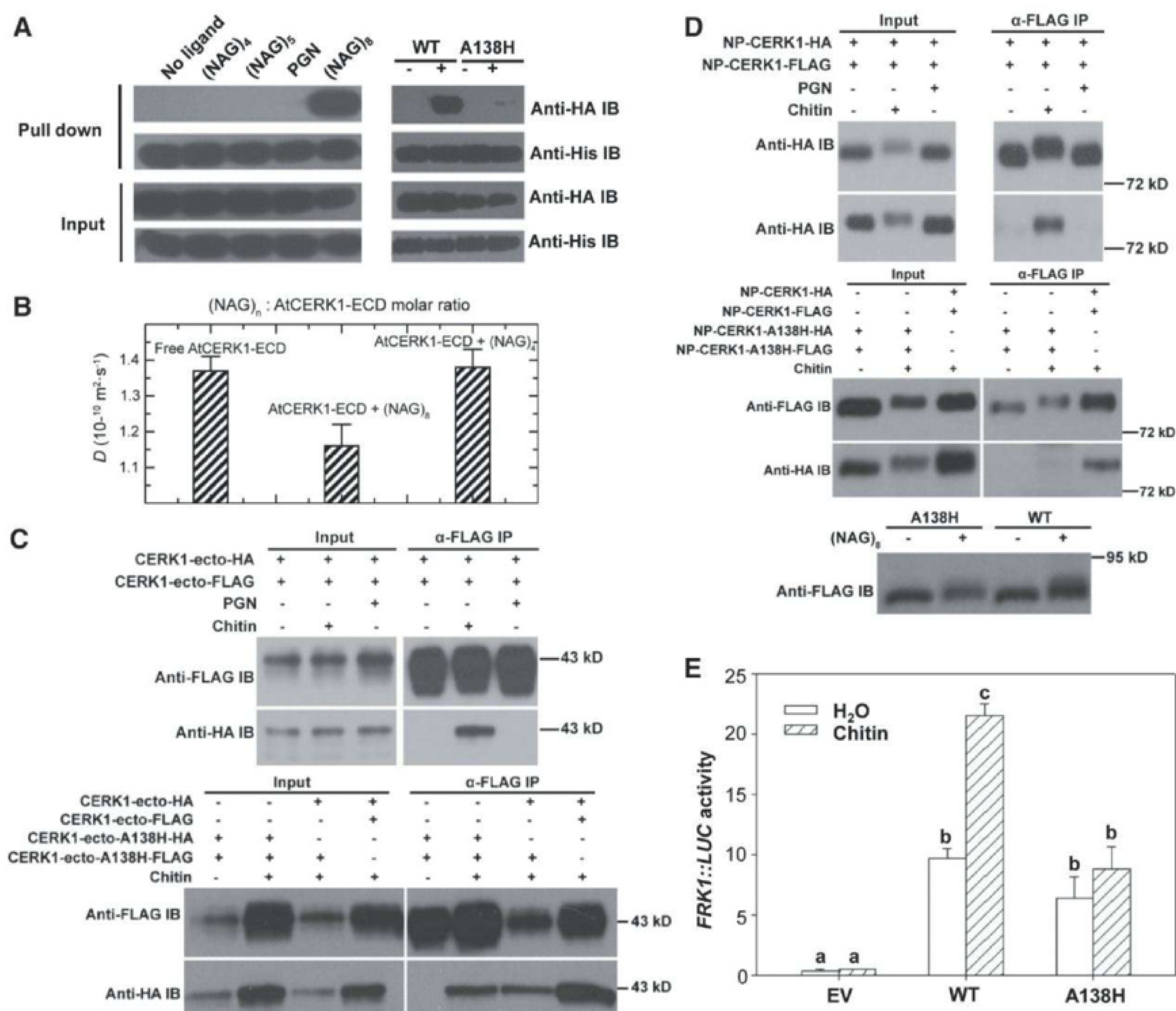
actions. Consistently, different chitin oligomers exhibited small differences in AtCERK1-ECD-binding activity as quantified by isothermal titration calorimetry (fig. S5). However, biological activity varies considerably among different chitin fragments, with highest activity for heptamers and octamers (22), and little (17) or no activity (23) for tetramers and pentamers, though the latter two can bind to AtCERK1 (17, 18). We hypothesized that higher oligomeric chitins like (NAG)₈ can simultaneously bind two or more AtCERK1 molecules and that the clustered AtCERK1 may be required for signaling.

To test the hypothesis, we examined the interaction between His₆- and hemagglutinin (HA)-tagged AtCERK1-ECD in the presence of different chitin oligomers or PGN using pull-down assay. In contrast with the shorter chitin

oligomers and PGN, (NAG)₈ induced a strong interaction between the HA-tagged and His₆-tagged AtCERK1-ECDs (Fig. 3A). Supporting the observations, our nuclear magnetic resonance (NMR) (Fig. 3B and figs. S6 and S7) and gel filtration assays (fig. S8) showed that (NAG)₈ but not (NAG)₄ or (NAG)₅ induced AtCERK1-ECD dimerization in solution. Substitution of Ala¹³⁸ (Fig. 2B) with the bulkier histidine is expected to circumscribe the chitin-binding site and interfere with chitin binding. Indeed, the mutant protein A138H exhibited a weakened (NAG)₅-binding activity (fig. S5) and compromised (NAG)₈-induced dimerization in pull-down (Fig. 3A) and gel filtration (fig. S8) assays. Consistent with the in vitro data, chitin (Fig. 3C) or (NAG)₈ (fig. S9), but not PGN, induced dimerization of AtCERK1-FLAG and AtCERK1-HA proteins lacking the intracel-

Fig. 3. Chitin-induced

AtCERK1-ECD dimerization is critical for signaling. **(A)** Chitin octamer induces AtCERK1-ECD dimerization in vitro. An equal amount of His₆- and HA-tagged AtCERK1-ECD proteins were mixed and supplemented with different chitin oligomers, as indicated, and PGN. After incubation, the mixture was loaded onto Ni-resin and then washed extensively. The eluted proteins were detected by Western blot with antibodies against His and HA. **(B)** (NAG)₈ but not (NAG)₄ induces AtCERK1-ECD oligomerization in solution. The translational diffusion coefficients *D* of free AtCERK1-ECD, and in the presence of excess (NAG)_{*n*}, were determined by pulsed field gradient-NMR diffusion experiments. The molar ratio of (NAG)_{*n*}: AtCERK1-ECD was 2:1. **(C)** Chitin induces AtCERK1-ECD dimerization in protoplasts. Top: HA- and FLAG-tagged AtCERK1-ECDs were co-expressed in wild-type (WT) *Arabidopsis* protoplasts. Coimmunoprecipitation assay was performed to detect chitin-induced dimerization after treatment with (+) or without (−) chitin (200 μg/ml). Bottom: A138H substitution attenuates chitin-induced AtCERK1-ECD dimerization. **(D)** Chitin induces dimerization of the full-length AtCERK1 protein in protoplasts. Top: HA- and FLAG-tagged AtCERK1 were coexpressed in WT *Arabidopsis* protoplasts under the control of native AtCERK1 promoter. Methods described in (C) were used to detect the proteins. Middle: A138H substitution attenuates chitin-induced dimerization of the full-length AtCERK1 protein. NP: native promoter. Bottom: A138H substitution attenuates (NAG)₈-induced band shift



of AtCERK1 protein in stable transgenic plants. Leaves of T1 transgenic plants were treated with H₂O or 60 μM (NAG)₈ for 15 min, and anti-FLAG immunoblot was used to detect band shift. **(E)** A138H attenuates chitin-induced FRK1::LUC expression. The *cerk1* mutant *Arabidopsis* protoplasts were transfected with empty vector (EV), AtCERK1 (WT), or AtCERK1-A138H (A138H) along with 35S::R-LUC and FRK1::LUC. The FRK1::LUC activity was determined after protoplasts were treated with chitin (200 μg/ml) or H₂O for 3 hours. Different letters above the bars indicate significant difference (mean + SD; *n* ≥ 3; *P* < 0.01).

lular kinase domain when coexpressed in protoplasts, which was reduced in the mutant protein A138H. Chitin-dependent dimerization was also observed for the wild-type AtCERK1 protein expressed under the control of native AtCERK1 promoter (NP-CERK1) but compromised in the mutant A138H protein (Fig. 3D). Notably, AtCERK1 overexpression resulted in chitin-independent AtCERK1 oligomerization and partial induction of *FRK1-LUC* (fig. S10), a reporter gene strongly induced by multiple PAMPs, including chitin (1). As previously reported (17), chitin induced a band shift of the AtCERK1 protein in protoplasts indicative of AtCERK1 phosphorylation, which surprisingly appeared not to be affected in the A138H mutant (Fig. 3D, middle). As demonstrated for flg22-induced FLS2 phosphorylation (24), ligand-induced phosphorylation of receptor kinases is a rapid process. Our protoplast-based assay may not be sensitive enough to distinguish the rapid phosphorylation in the wild-type AtCERK1 and the slow phosphorylation in the A138H mutant protein. Thus, although less effectively, the mutant protein was still fully phosphorylated at the time of detection. Consistently, (NAG)₈-induced AtCERK1 phosphorylation, which is less efficient than that by chitin (17), was reduced in stable transgenic plants expressing A138H (Fig. 3D, bottom). As previously reported (5), the *cerk1* mutant protoplasts were unable to activate *FRK1-LUC* transcription when induced with chitin (Fig. 3E).

Introduction of the wild-type AtCERK1 but not the mutant A138H construct into the *cerk1* protoplasts led to increased *FRK1-LUC* expression, which was enhanced by chitin. Together, our data support the idea that chitin-induced cross-linking of AtCERK1 activates downstream signaling.

Given that (NAG)₅ bound to the AtCERK1-ECD but failed to induce its oligomerization, it was expected to interfere with (NAG)₈- or chitin-induced AtCERK1 oligomerization. As anticipated, (NAG)₅ attenuated (NAG)₈-induced AtCERK1-ECD dimerization in vitro in a dose-dependent manner (Fig. 4A). Similar results were obtained for AtCERK1-ECD expressed in protoplasts (Fig. 4B). Furthermore, (NAG)₅ reduced (NAG)₈-induced expression of *FRK1-LUC* in the wild-type protoplasts (Fig. 4C). We reasoned that if the chitin-induced AtCERK1 dimerization is required for signaling, overexpression of AtCERK1-ECD (OXLysM) would result in nonproductive dimerization and signaling blockage. Indeed, OXLysM led to its heterodimerization with full-length AtCERK1 in protoplasts and abolished chitin-induced expression of *FRK1-LUC* and band shift of AtCERK1 protein (Fig. 4C). (NAG)₈-induced accumulation of H₂O₂ in wild-type *Arabidopsis* plants was progressively reduced with increasing (NAG)₅ (Fig. 4D). Further supporting the dominant interactions of three NAGs with AtCERK1-ECD (Fig. 2B), the chemically synthesized compound Ch-2 with a linker connect-

ing two (NAG)₃ (fig. S11) moderately induced AtCERK1-ECD dimerization in vitro (Fig. 4E), compared to a lower activity of Ch-1, and in protoplasts though less effectively than (NAG)₈ (Fig. 4F).

Our biochemical and molecular data show that (NAG)₈ can act as a bivalent ligand to induce cross-linking of AtCERK1-ECD required for chitin-induced signaling. The low chitin-binding activity of the isolated AtCERK1-ECD in solution may translate into tighter interactions in intact plant cells because in vivo the other part(s) of the full-length protein could stabilize the chitin-induced AtCERK1-ECD association. Additionally, anchoring to the two-dimensional lipid bilayer might limit diffusion of the AtCERK1 protein. Chitin-induced oligomerization was also found in the LysM-containing proteins ECP6 (25) and Hevein (26). A minimum length of chitin oligomers is expected for binding two AtCERK1-ECDs. It is unlikely that two AtCERK1-ECDs bind to (NAG)₆ with each peptide associating with three NAGs, as this would cause steric interactions between the two peptides. However, we cannot rule out the possibility that (NAG)₆ interacts with two AtCERK1-ECDs in an asymmetric mode, leading to weaker AtCERK1-ECD dimerization. Although (NAG)₇ and (NAG)₈ display the highest biological activity among the chitin oligomers tested, it remains unknown whether there exist longer chitin oligomers better optimized in length

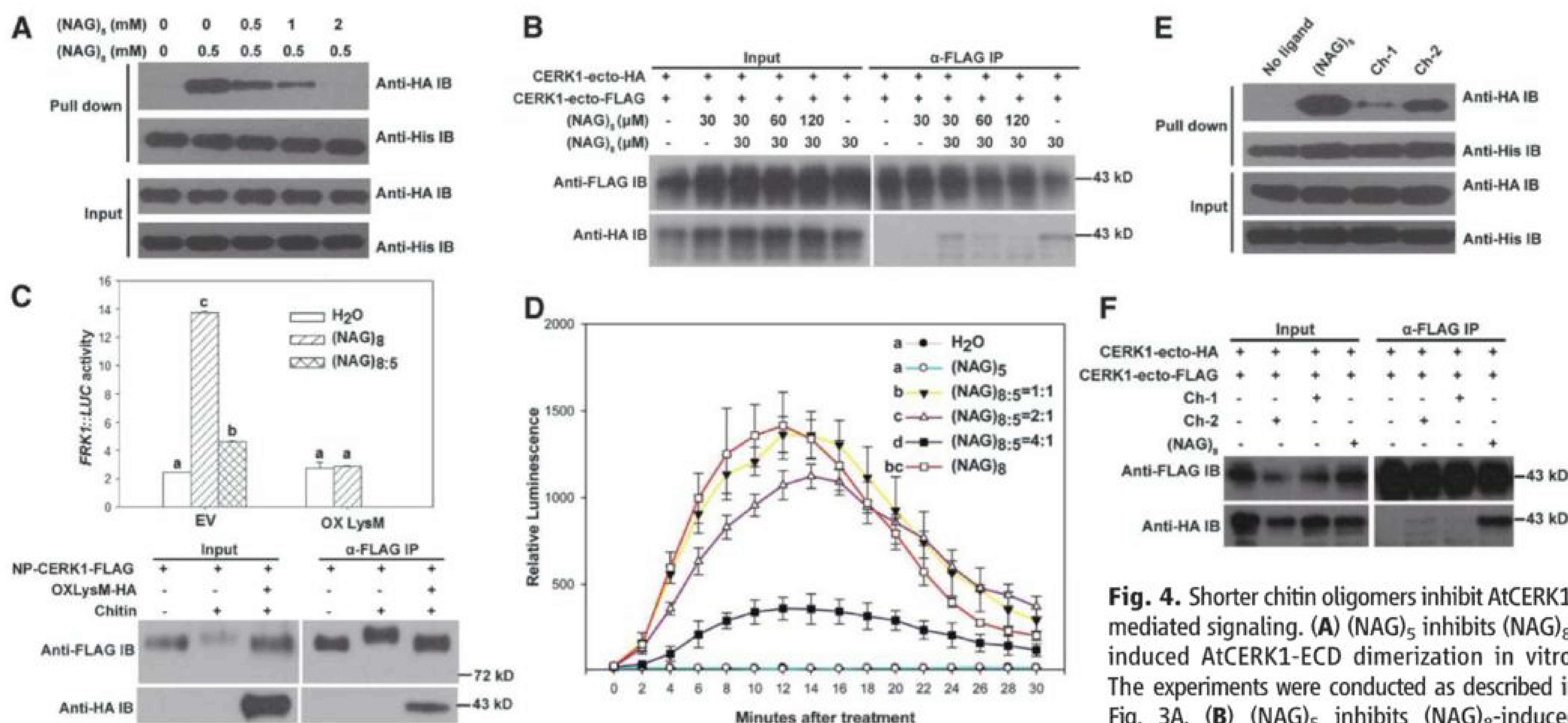


Fig. 4. Shorter chitin oligomers inhibit AtCERK1-mediated signaling. (A) (NAG)₅ inhibits (NAG)₈-induced AtCERK1-ECD dimerization in vitro. The experiments were conducted as described in Fig. 3A. (B) (NAG)₅ inhibits (NAG)₈-induced AtCERK1-ECD dimerization in protoplasts. HA- and FLAG-tagged AtCERK1-ECDs were coexpressed in WT *Arabidopsis* protoplasts, treated with H₂O (–) and chitin oligomers, as indicated, for 10 min, and coimmunoprecipitation assay was conducted to detect the dimerization. (C) (NAG)₅ and overexpression of AtCERK1-ECD (OXLysM) inhibit chitin-induced signaling. Top: Chitin induces heterodimerization between the overexpressed AtCERK1-ECD and the full-length AtCERK1. Bottom: OXLysM inhibits chitin-induced *FRK1::LUC* activity. WT *Arabidopsis* protoplasts were transfected with an empty vector (EV) or OXLysM plasmids along with *FRK1::LUC* and *35S::R-LUC*. The activity of *FRK1::LUC* was analyzed after treatment with 60 μ M (NAG)₈ or a mixture of 60 μ M (NAG)₈ and 240 μ M (NAG)₅ [(NAG)_{8:5}] for 3 hours. Different letters above the bars indicate significant difference (mean $\pm$ SD; $n \geq 3$ replicates; $P < 0.01$). (D) (NAG)₅ inhibits (NAG)₈-induced H₂O₂ production in planta. WT *Arabidopsis* leaves were treated with 30 μ M (NAG)₈ or a mixture of 30 μ M (NAG)₈ and (NAG)₅ with different ratios [(NAG)_{8:5}] as indicated (mean $\pm$ SD; $n \geq 4$; $P < 0.01$). (E) A synthetic chitin derivative induces AtCERK1-ECD dimerization in vitro. The experiments were performed as described in Fig. 3A. (F) AtCERK1-ECD dimerization in protoplasts in response to synthetic chitin derivatives. The experiments were performed as described in Fig. 3C.

and rigidity to allow more efficient AtCERK1 activation. The free hydroxyl groups at C4 and C1 (Fig. 2B) from the two ends of (NAG)₄ are not involved in AtCERK1-ECD binding, suggesting that chitin oligomers with more than eight units can interact with more than two AtCERK1 molecules simultaneously. Consistently, AtCERK1 interacts strongly with polymeric chitin (17) because of multiple AtCERK1-binding sites.

Like the mammalian receptor tyrosine kinases (RTKs) and toll-like receptors (TLRs), plant RLKs have emerged as critical regulators of key cellular processes. Structural analyses have demonstrated that ligand-induced dimerization is a common theme for the activation of RTKs (27) and TLRs (28). The molecular mechanisms underlying RLK activation, however, remain much less understood. Ligand-induced heterodimerization has been reported for several RLKs (29–32). Our results suggest that chitin-mediated cross-linking of AtCERK1s is required for immune signaling, shedding light on a molecular mechanism of ligand-induced RLK activation.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6085/1160/DC1
Materials and Methods

Table S1

Figs. S1 to S11

References (33–40)

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Rocket Launcher Mechanism of Collaborative Actin Assembly Defined by Single-Molecule Imaging

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Interacting sets of actin assembly factors work together in cells, but the underlying mechanisms have remained obscure. We used triple-color single-molecule fluorescence microscopy to image the tumor suppressor adenomatous polyposis coli (APC) and the formin mDia1 during filament assembly. Complexes consisting of APC, mDia1, and actin monomers initiated actin filament formation, overcoming inhibition by capping protein and profilin. Upon filament polymerization, the complexes separated, with mDia1 moving processively on growing barbed ends while APC remained at the site of nucleation. Thus, the two assembly factors directly interact to initiate filament assembly and then separate but retain independent associations with either end of the growing filament.

Regulation of actin assembly is a fundamental requirement in all eukaryotic cells, and a growing number of factors have been identified that either inhibit or promote this process. For example, the combined presence of the actin monomer-binding protein profilin and the filament end-binding capping protein (CapZ) strongly suppresses both spontaneous filament nucleation and elongation. Thus, filament assembly in vivo requires nucleation and elongation factors to overcome these barriers

to assembly (1–3). The formation of most cellular actin structures depends on two or more such factors, which often interact directly. A formin is a component of many actin assembly-promoting factor (APF) pairs that likely function together in vivo: the formins FMN/Cappucino (Capu) and Spire (4), the formin Bni1 and Bud6 (5), the formin mDia1 and adenomatous polyposis coli (APC) (6), and the formin dDia2 and *Dictyostelium* vasodilator-stimulated phosphoprotein (DdVASP) (7).

The dimeric formin-homology 2 (FH2) domain of formins processively tracks the growing barbed end of the actin filament, protecting it from capping proteins (8–10). Adjacent FH1 domains recruit profilin-actin complexes and can increase the rate of elongation at barbed ends

(11). Whereas profilin enhances formin-mediated filament elongation, its presence also strongly suppresses filament nucleation by formins (12). Collaboration of formins with other APFs that bind multiple actin monomers (4–6, 13) may be required to overcome the inhibitory effects of profilin and capping protein. However, direct evidence for this hypothesis has been lacking. To address this, we reconstituted mDia1-APC-mediated actin assembly with purified, fluorescently tagged proteins and used multiwavelength single-molecule TIRFM (total internal reflection fluorescence microscopy) to directly visualize and define the mechanisms promoting collaborative filament assembly.

For single-molecule imaging, we purified a soluble, modified *O*⁶-alkylguanine-DNA alkyltransferase (AGT)-tagged (also known as SNAP-tag) C-terminal fragment of APC (APC-C): residues 2130–2843, encompassing its “Basic” domain, which is sufficient to mediate actin nucleation, and the domain that binds EB1 (a microtubule end-binding protein) (6, 14). SNAP-APC-C labeled with SNAP-surface-647 (herein named SNAP-647-APC-C) displayed activities identical to those of APC-C in pyrene-actin assembly assays (fig. S1A). Photobleaching data suggested that most SNAP-647-APC-C molecules are dimeric (fig. S1, B and C; and movie S1), consistent with hydrodynamic studies on maltose-binding protein-tagged APC-C (6).

APC, like Spire and Bud6, has been proposed to catalyze actin nucleation by binding actin monomers to form an F-actin seed (4–6). We used dual-color TIRFM to directly visualize surface-adsorbed SNAP-647-APC-C molecules, appearing as discrete spots, during the assembly

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of Oregon Green (OG)–actin filaments (Fig. 1A and movie S2). From some of these spots, we observed actin filaments emerge and grow primarily from their barbed ends, and APC did not alter the growth rate in the presence or absence of profilin (fig. S2A). Further, APC-C did not block polymerization at pointed ends (fig. S2B), which suggests that it stays bound to the filament at the site of nucleation.

For the majority of surface-tethered filaments (88 out of 106 observed), APC fluorescence remained visible at the nucleation site 10 min after nucleation, which suggested a thermodynamically stable association. We also observed filaments with APC-C associated at their ends freely diffusing near the surface, which suggested that the stable association is not due to surface tethering. At concentrations ≥ 10 nM SNAP-647-APC-C, we occasionally observed APC-C accumulating on filament sides, which led to bundling (fig. S3 and movie S3), consistent with previous reports (15).

Quantifying the oligomeric state of APC-C at the onset of nucleation suggested that an APC-C dimer was sufficient to nucleate filament assembly, although a minority of the filaments originated from spots consisting of more than

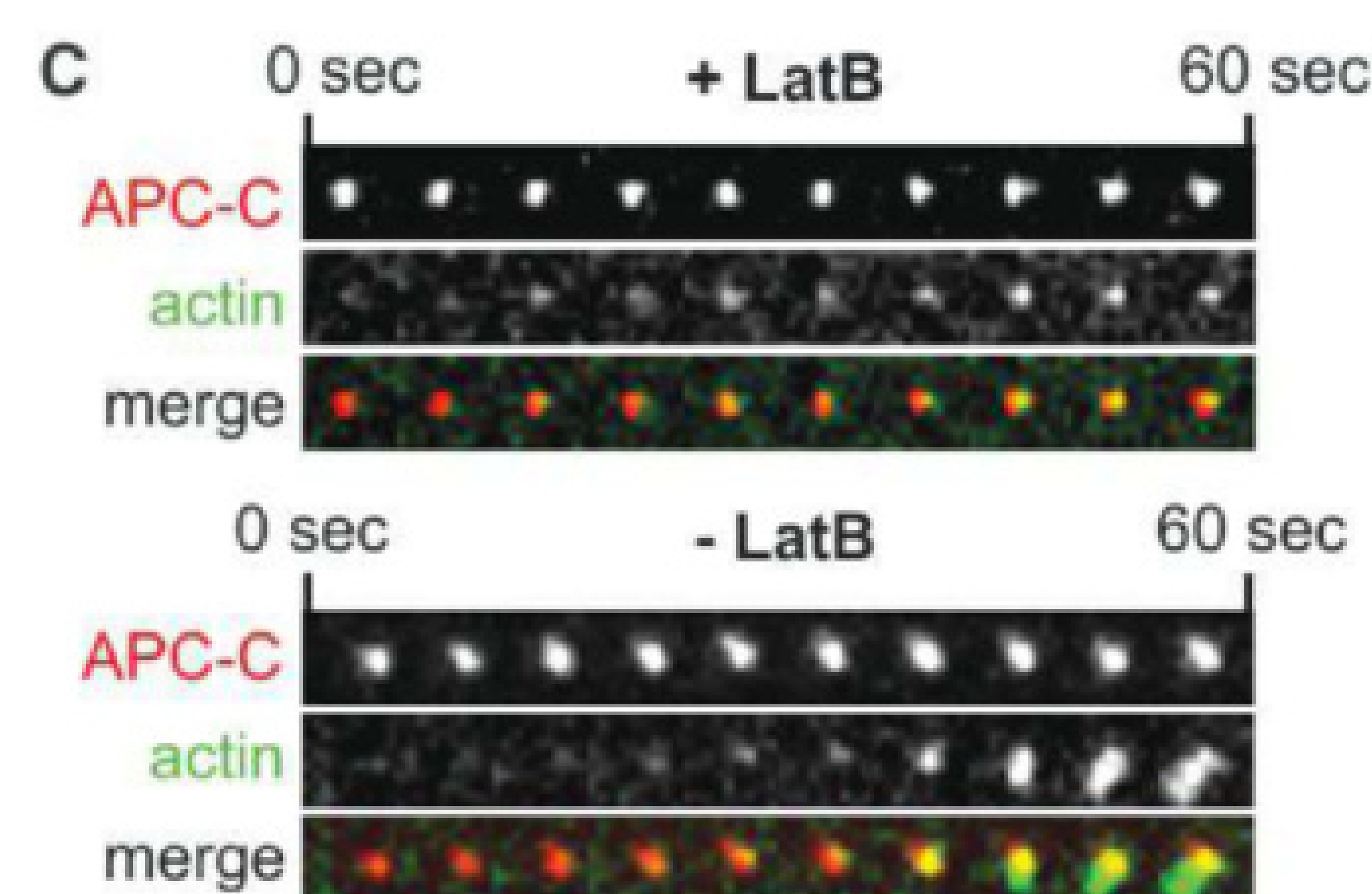
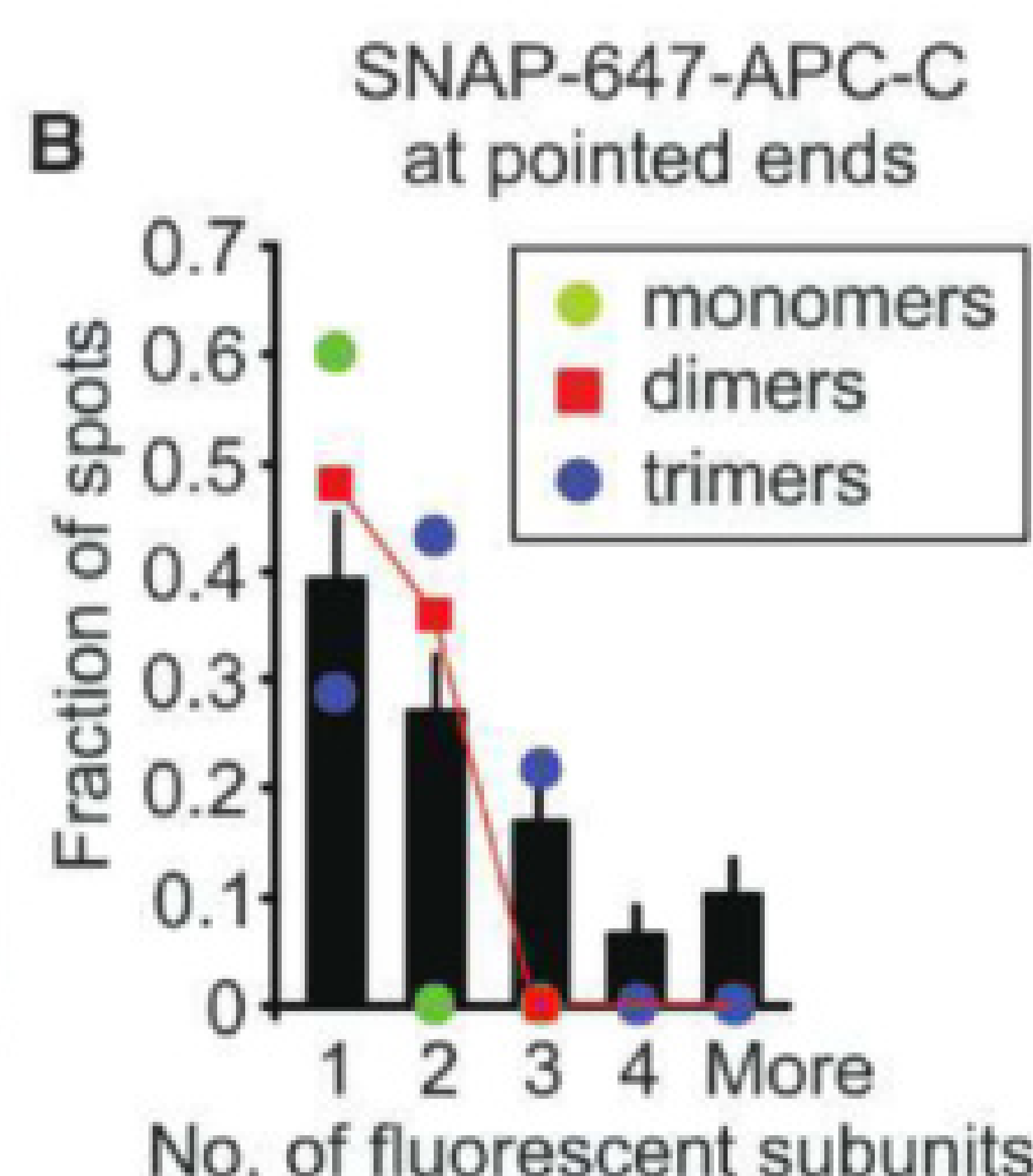
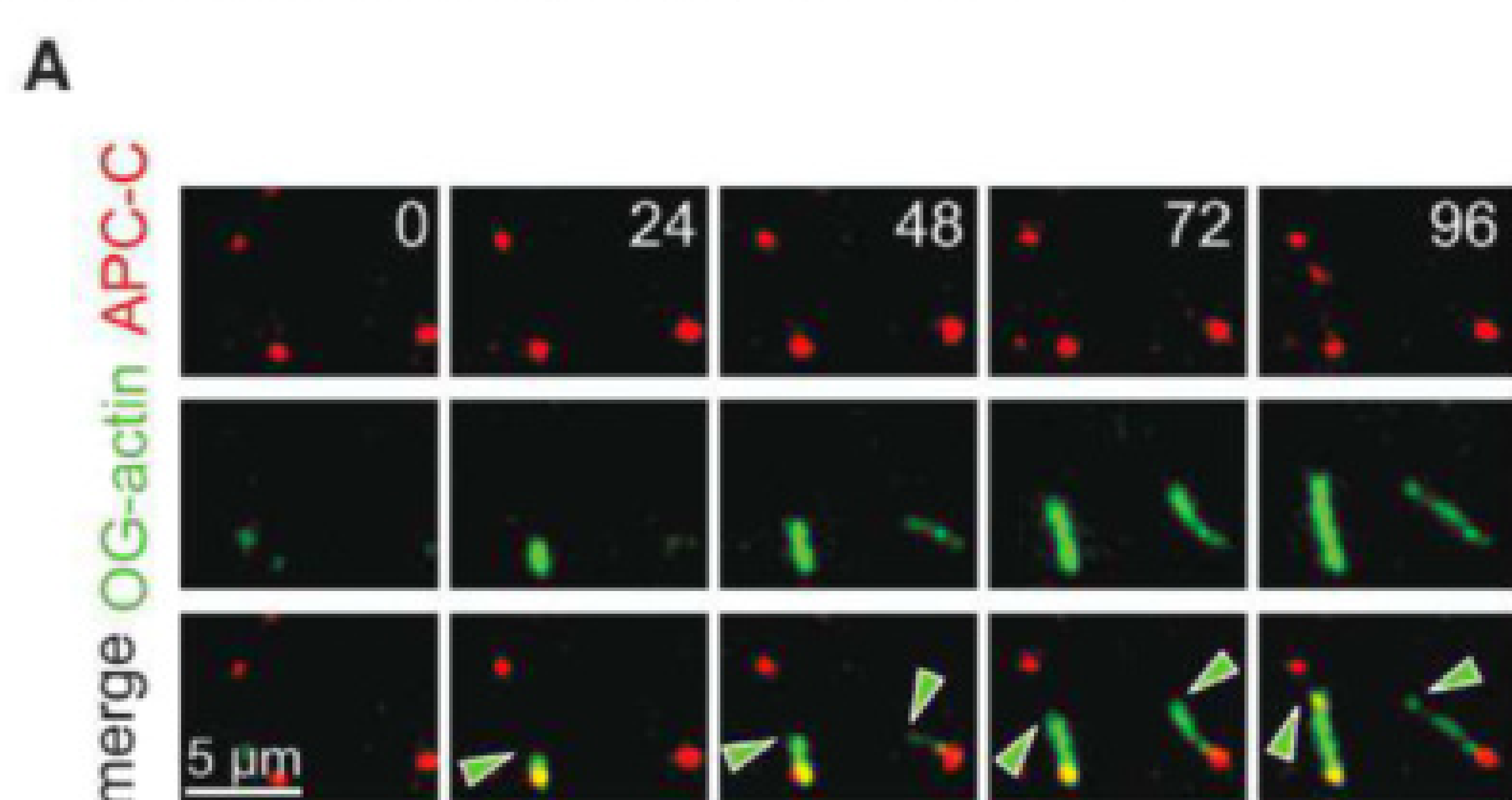
two APC-C subunits (Fig. 1B). When we included latrunculin B (latB), which associates with actin monomers and blocks polymerization, OG-actin still accumulated in some of the SNAP-647-APC-C spots; however, no filaments polymerized (Fig. 1C and movie S4). These observations suggest that nucleation by APC involves monomer recruitment, as opposed to capture of spontaneously formed F-actin intermediates.

We next purified SNAP-tagged mDia1-C—which contains FH1, FH2, and diaphanous autoregulatory (DAD) domains—and fluorescently labeled it (SNAP-549-mDia1-C). Single-molecule photobleaching experiments suggested that the SNAP-549-mDia1-C preparation is predominantly dimeric but also contains higher-order oligomers (fig. S4, A and B). SNAP-549-mDia1-C and unlabeled mDia1-C stimulated indistinguishable rates of pyrene-actin assembly (fig. S4C). Using dual-color TIRFM, we observed single SNAP-549-mDia1-C molecules translocating with the growing barbed ends of filaments both in the presence and absence of profilin (Fig. 1, D and E; fig. S5; and movies S5, S6, and S7). Processive association of the formin was verified by additional lines of evidence (figs. S5 and S6

and movie S7). mDia1-C and SNAP-549-mDia1-C each elongated filaments at equivalent rates in presence and absence of profilin (Fig. 1F). Similar results were obtained with SNAP-549-mDia1-C in the presence of 1 nM CapZ. Our observations directly confirm that individual formin molecules processively track filament barbed ends, with or without profilin, and accelerate elongation in a profilin-dependent manner (11).

APC binds mDia formins in solution (16). Therefore, we used dual-color TIRFM to investigate interactions between SNAP-647-APC-C and SNAP-549-mDia1-C molecules. After mixing the two labeled proteins in solution, we examined the composition of complexes that adsorbed to the surface. We found that $51 \pm 2.3\%$ (SEM) of SNAP-549-mDia1-C spots colocalized with SNAP-647-APC-C spots (Fig. 2A). Coincidental colocalization was minimal (1.7%) (14). Taking into account the labeling stoichiometries of both proteins, our analysis is consistent with a model in which most of the fluorescent spots contain two SNAP-647-APC-C and two SNAP-549-mDia1-C molecules (Fig. 2, A and B). Similar results were obtained in the presence of latB-sequestered actin monomers [$60 \pm 3.9\%$ (SEM)

Actin assembly by SNAP-647-APC-C



Actin assembly by SNAP-549-mDia1-C

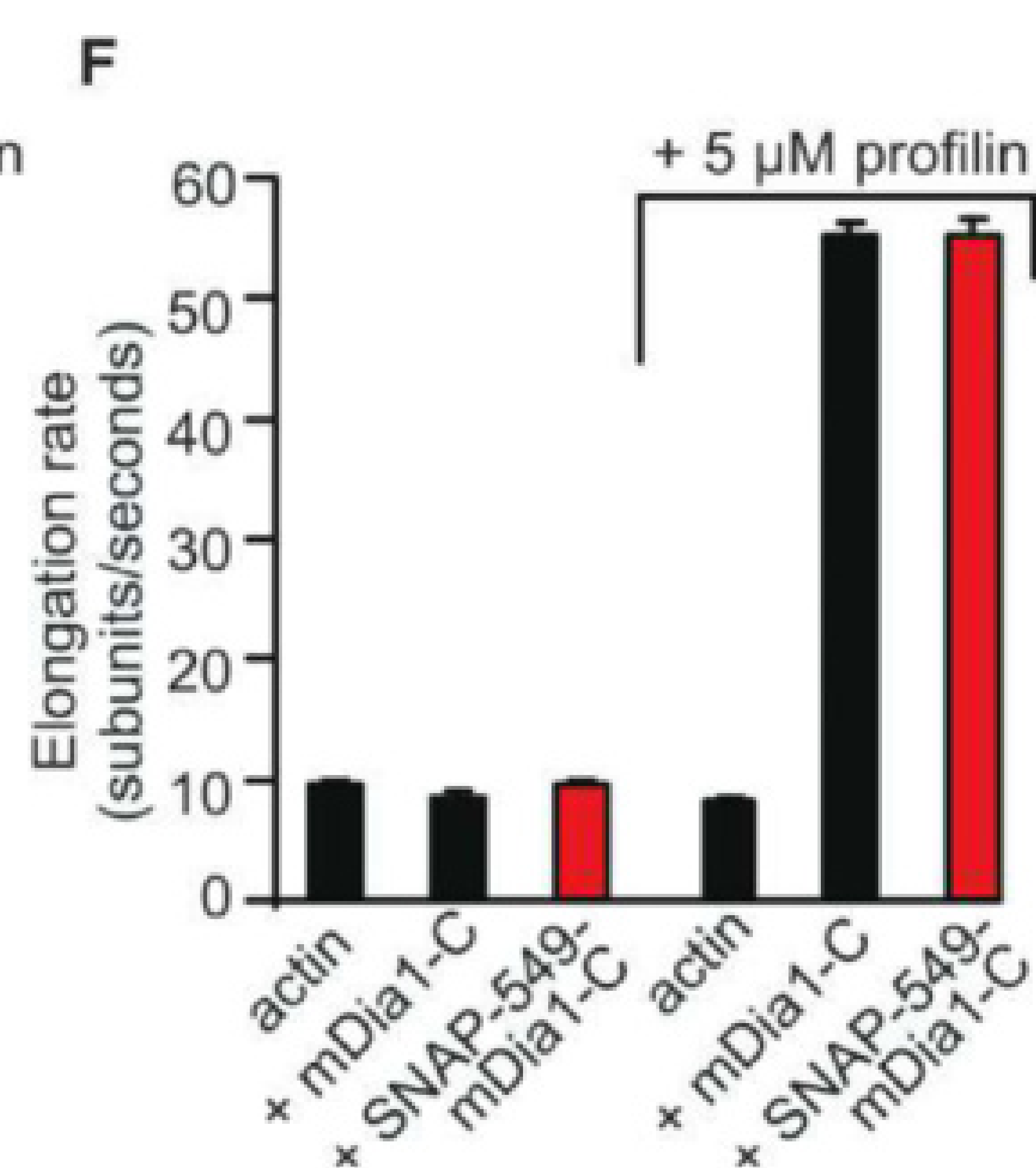
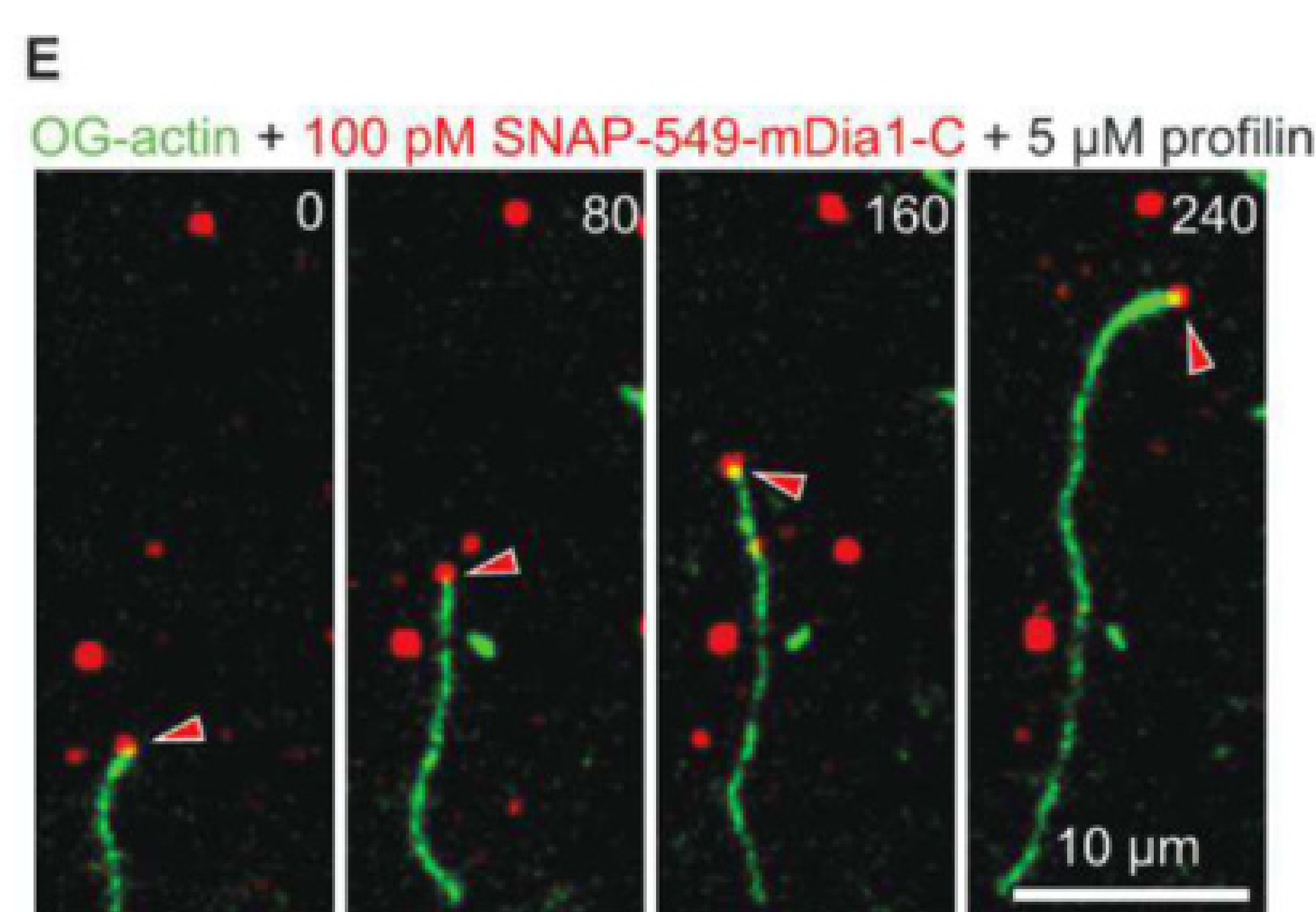
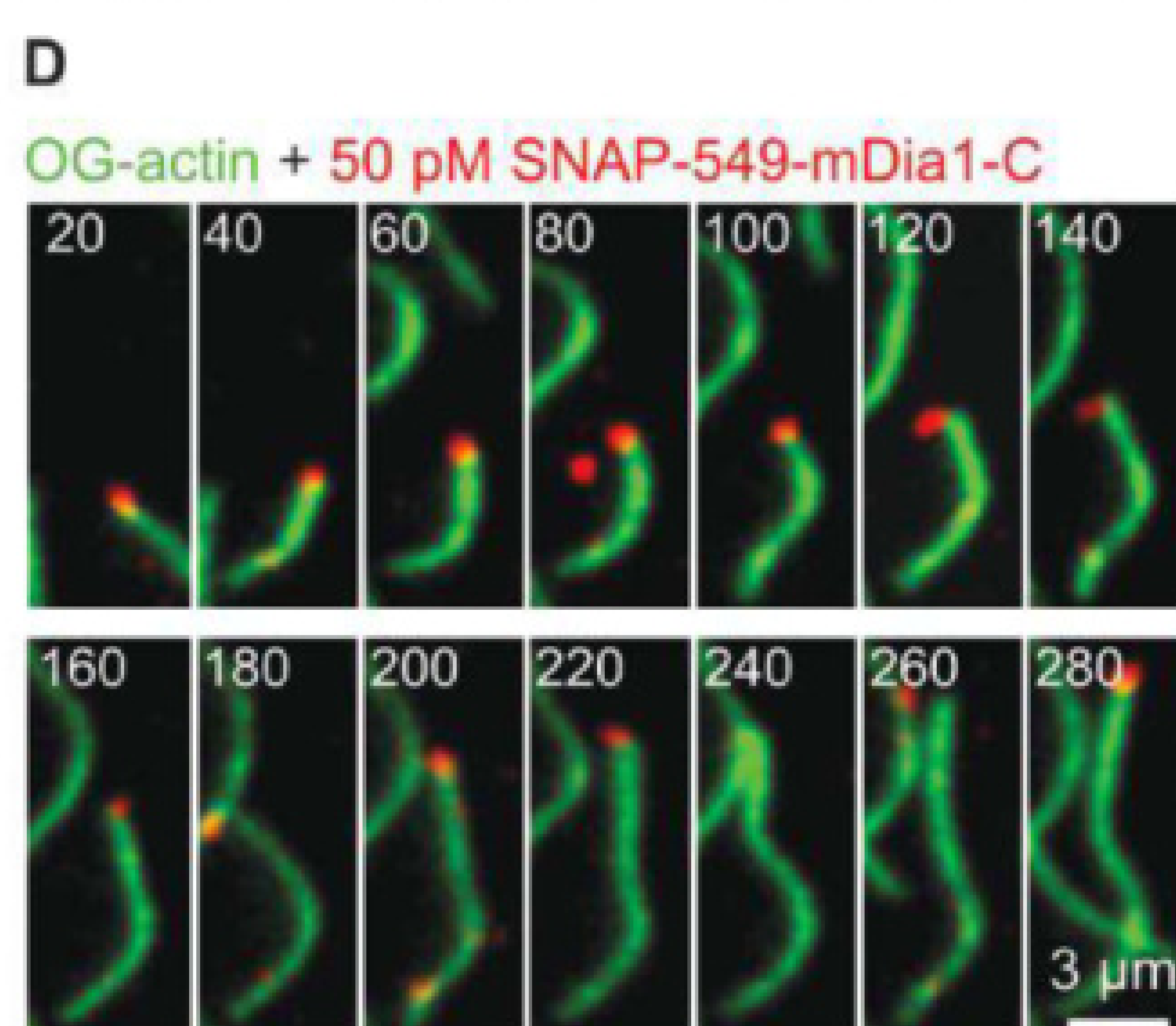


Fig. 1. Single-molecule analysis of the APC-C– and mDia1-C–mediated actin assembly processes. **(A)** Dual-color TIRFM of OG-actin filaments (1 μ M, 10% labeled, green) originating from SNAP-647-APC-C spots (10 nM, red). Green arrowheads mark the growing barbed ends. **(B)** Fluorescence intensity analysis of number of SNAP-647-APC-C subunits at filament ends. **(C)** Micrographs of OG-actin accumulation at SNAP-647-APC-C spots in the absence and presence

of latB. **(D and E)** Dual-color TIRFM of OG-actin filaments (1 μ M, 10% labeled, green) being elongated by single SNAP-549-mDia1-C molecules (red) without **(D)** and with **(E)** profilin. Time is given in seconds. Red arrowheads in **(E)** mark the formin molecule associated with the barbed end. **(F)** Average elongation rates of mDia1-C– and SNAP-549-mDia1-C–assembled filaments without and with profilin. Error bars represent SE, $n \geq 12$, $N = 3$.

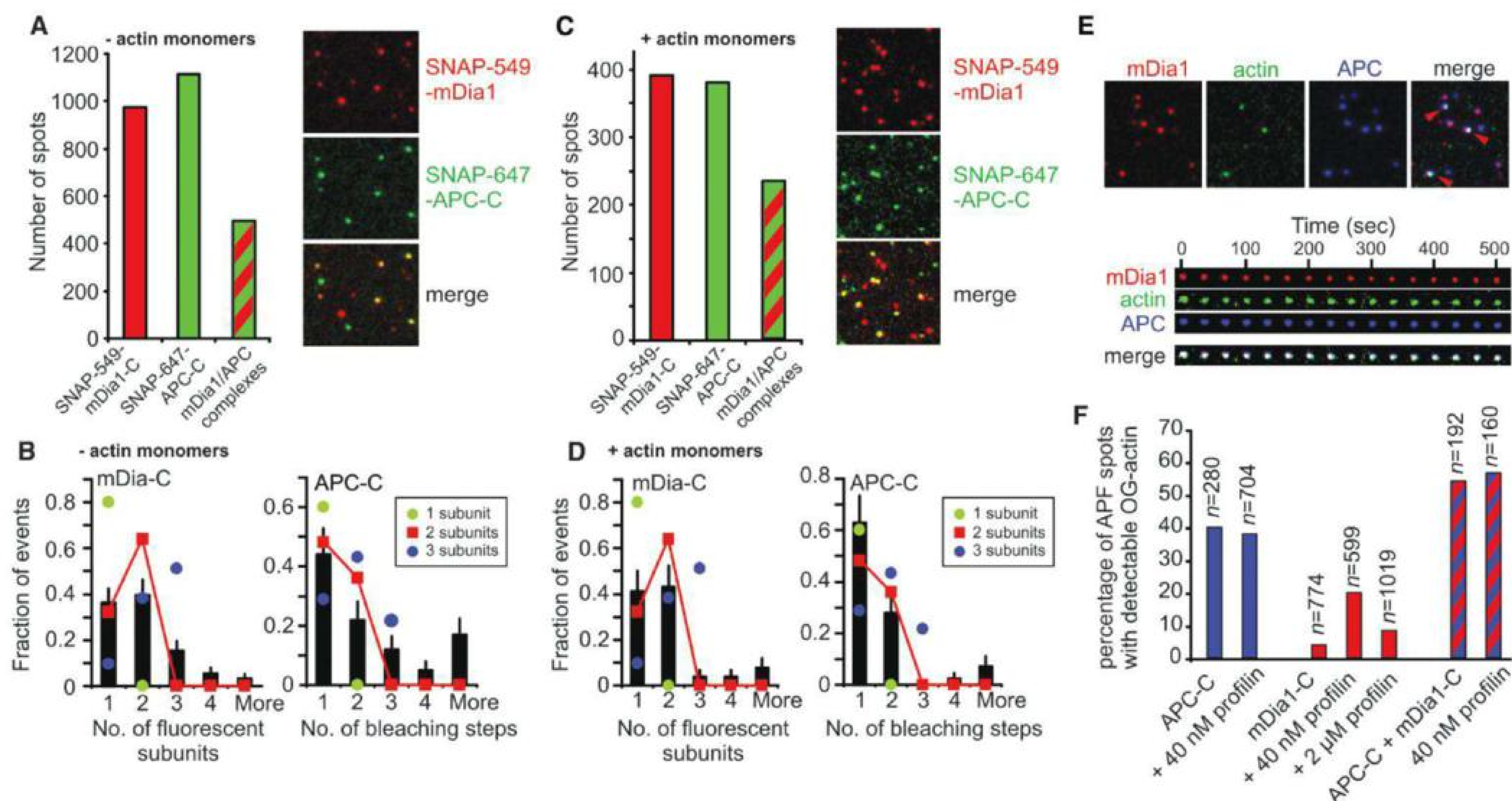


Fig. 2. Association of APC-C and mDia1-C and formation of tripartite complexes with G-actin. **(A)** Colocalization of SNAP-647-APC-C and SNAP-549-mDia1-C in surface-adsorbed complexes. The proteins (200 nM each) were preincubated for 10 min, diluted 200-fold, and visualized by TIRFM. Spots emitting fluorescence from SNAP-549, SNAP-647, or both were counted. **(B)** Oligomeric state of APC-C-mDia1-C complexes ($n = 91$) determined by fluorescence intensity analysis for mDia1-C and photobleaching step analysis for APC-C (see methods). **(C and D)** Same as **(A)** and **(B)**, except that 20 μ M

latB-sequestered actin monomers were included during preincubation (100 nM in final reaction). In **(D)**, $n = 43$. Error bars represent SE. **(E)** Tripartite complexes formed by preincubating 200 nM SNAP-647-APC-C, 200 nM SNAP-549-mDia1-C, or 8 μ M latB-OG-actin and then diluting 200-fold before surface adsorption. Field-of-view (top) and time-lapse record of a single spot containing all three proteins (bottom). **(F)** Fraction of APC-mDia1 spots that contain detectable OG-actin in the presence or absence of profilin (see the supplementary materials).

colocalization] (Fig. 2, C and D). These data suggest that actin monomers neither interfere with APC-formin interactions nor change the oligomeric state of the complex and may even modestly promote APC-formin interactions. Using triple-color TIRFM, we observed $54.8 \pm 6.6\%$ (SEM) of APC/mDia1 complexes stably occupied by latB-OG-actin (Fig. 2, E and F). These assemblies may represent nucleation intermediates that are normally short-lived during filament assembly but can be trapped by latB. In the absence of mDia1, OG-actin accumulation was observed in $40.5 \pm 4.6\%$ (SEM) of SNAP-647-APC-C spots, but in the absence of APC-C, OG-actin accumulation was observed in only $4.0 \pm 0.7\%$ (SEM) of SNAP-549-mDia1-C spots (Fig. 2F). These observations suggest that APC-C is the primary actin monomer-recruiting factor in the APC-mDia1 complex. Profilin did not change OG-actin accumulation in SNAP-647-APC-C spots but resulted in an increase in OG-actin accumulation to $20.2 \pm 1.8\%$ (SEM) in SNAP-549-mDia1 spots, which likely represents profilin-actin recruitment by FH1 domains. Consistent with this view, competition with a 50-fold excess of profilin over OG-actin reduced the number of formin spots, with OG-actin accumulation to $8.5 \pm 0.9\%$ (SEM).

Next, we used triple-color TIRFM to define the sequence of events and spatial locations of SNAP-549-mDia1-C and SNAP-647-APC-C during collaborative filament assembly. We included CapZ and profilin to reconstitute cellular barriers to actin assembly. Tripartite complexes consisting of SNAP-549-mDia1-C, SNAP-647-APC-C, and OG-actin were observed (asterisk in Fig. 3A). Filaments grew from some of these spots. Concomitant with filament growth, APC and mDia1 invariably separated ($n > 100$), which left SNAP-647-APC-C at the pointed end and SNAP-549-mDia1-C translocating on the growing barbed end (Fig. 3A, fig. S8, and movie S8). The same was observed in the absence of profilin (fig. S9 and movie S9). Most nucleation complexes had already separated by the start of imaging, as indicated by the presence of many short filaments carrying SNAP-549-mDia1-C at their barbed ends and SNAP-647-APC-C at their pointed ends (fig. S10). Note that SNAP-647-APC-C molecules were never seen bound to processively moving formins ($n > 100$), which suggested that mDia1 molecules in the process of catalyzing barbed-end polymerization may not be capable of interacting with APC-C. Filaments bearing APC-C on one end and mDia1 on the other end showed rates of elongation indistin-

guishable from rates observed for mDia1-C without APC-C (fig. S11). Thus, elongation of filaments is likely to be purely mDia1-catalyzed with no contribution from APC.

Increasing concentrations of APC-C produced increasing numbers of SNAP-549-mDia1-C-elongated filaments in the presence of profilin and CapZ (Fig. 3B and fig. S12A). Quantitatively similar results were obtained using unlabeled APC-C and mDia1-C (Fig. 3C). Moreover, longer preincubation of mDia1-C and APC-C increased the number of mDia1-C-elongated filaments by about another twofold (fig. S12B). These effects of APC-C were less pronounced in the absence of profilin (Fig. 3B), which suggested that APC-C plays a key role in overcoming the nucleation barrier imposed by profilin.

Two other APFs (Spire and Bud6) were recently shown to bind to formin C-terminal tail sequences (4, 5, 13, 17). Similarly, we found that APC-C-coated beads depleted soluble mDia1-C but not mDia1-FH1FH2 (which lacks tail sequences) from supernatants (Fig. 3D), which suggests that APC binds mDia1 tail sequences. Moreover, APC-C failed to increase actin assembly activity in combination with mDia1-FH1FH2 (red bars, Fig. 3C and fig. S13). These observations strongly suggest that direct interactions between APC and mDia1

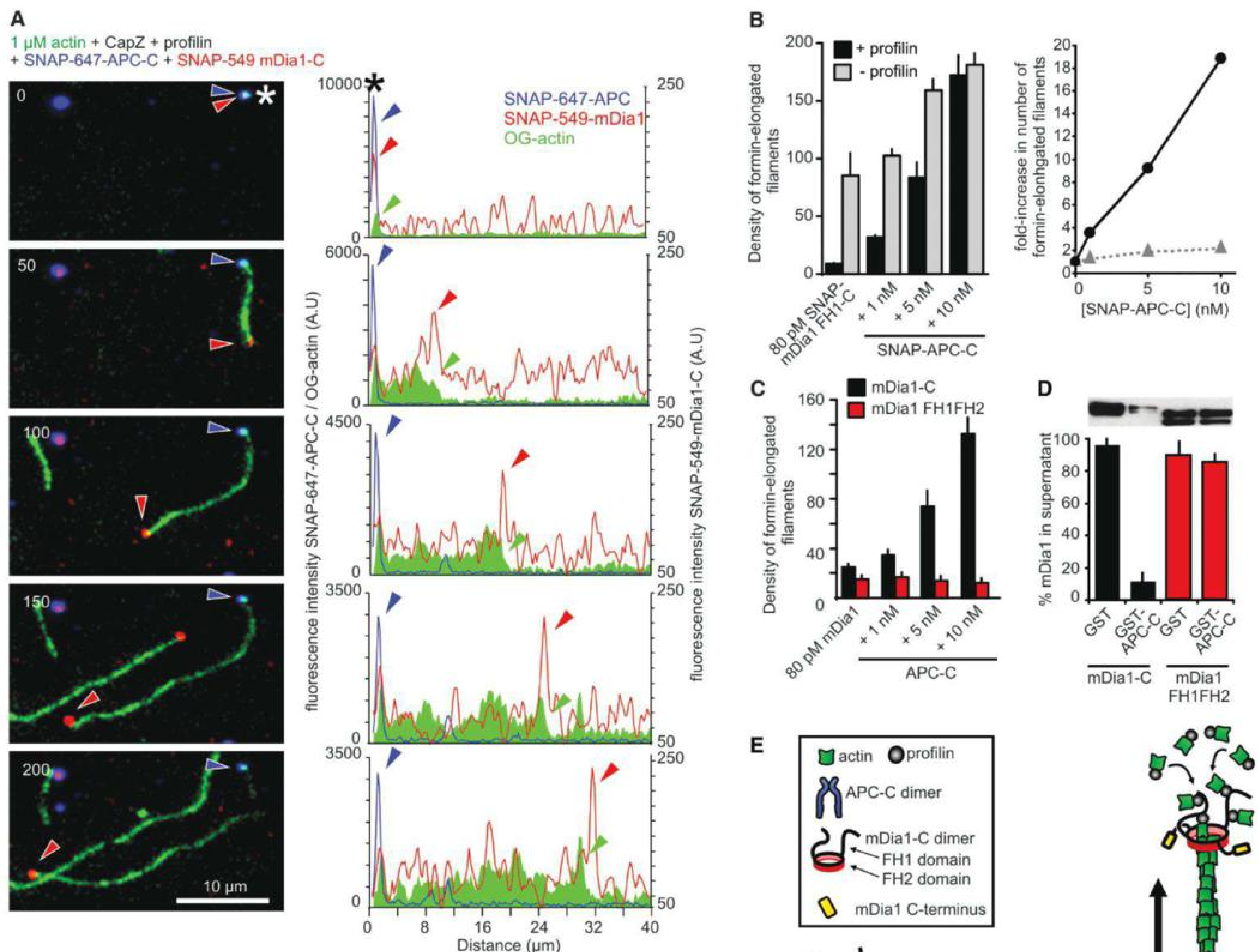


Fig. 3. Single-molecule visualization of APC-C and mDia1-C collaborating to assemble actin filaments. **(A)** Triple-color TIRFM of the assembly of 1 μ M OG-actin (green) in the presence of 50 pM SNAP-549-mDia1-C (red), 5 nM SNAP-647-APC-C (blue), 1 nM CapZ, and 5 μ M profilin. Asterisk indicates APC-mDia1-actin nucleation complex. Images (left; time in seconds) and corresponding profiles of fluorescence intensity along the length of the filament (right). Arrowheads mark the barbed end (green), mDia1 (red), and APC (blue). **(B)** (Left) Density of formin-elongated filaments per 100- $\times$ 100- μ m area for reactions containing 80 pM SNAP-549-mDia1-C, 1 nM CapZ, or variable concentrations of SNAP-APC-C, $\pm$ 5 μ M profilin. Error bars represent SD, $N = 3$. (Right) APC-C-dependent fold increase in density of formin-elongated filaments in the presence (black) and absence (gray) of profilin. **(C)** Same as (B), except using non-SNAP-tagged APC-C, mDia1-C and tail-less mDia1 FH1FH2. **(D)** Western blot with antibody against the six-histidine tag (6His) showing levels of mDia1-C and mDia1 FH1FH2 (both tagged with 6His) in supernatants after depletion by glutathione *S*-transferase (GST)-tagged (control) or GST-APC-C beads. Error bars represent SD, $N = 2$. **(E)** Proposed "rocket launcher" model for mDia1-APC collaboration during actin filament assembly (details in text).

tail sequences are required for their collaborative effects in actin assembly.

Taken together, our results suggest a mechanism (Fig. 3E) in which APC-C dimers recruit actin monomers and bind the tail region of mDia1-C to form a tripartite nucleation complex. At the onset of actin polymerization, the complex separates, leaving APC-C stably associated near the pointed end, while mDia1-C is propelled away on the rapidly growing barbed end (Fig. 3E). By this mechanism, APC plays the central role in assembling the nucleation seed, and the formin

then detaches and serves as the elongation catalyst. It remains to be seen whether the same mechanism applies to other pairs of APFs that are known to interact, e.g., Spire with Capu and Bud6 with Bni1 (4, 5). This work may have important implications for APC biological function. Full-length APC is a large protein that binds to numerous other cellular factors and functions in a wide variety of regulatory pathways (18). Thus, the mechanism for APC-mDia1 collaboration described here may result in site-specific actin assembly and, possibly, tethering of polymerizing actin filaments

at their pointed ends to APC-binding partners (e.g., IQGAP1, microtubule plus ends) (19, 20). These phenomena may serve to promote directed cell migration or other functions that require coordinated reorganization of the actin cytoskeleton with respect to other cellular structures.

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Supplementary Materials

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Figs. S1 to S13

References (21–31)

Movies S1 to S9

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The Amyloid Precursor Protein Has a Flexible Transmembrane Domain and Binds Cholesterol

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C99 is the transmembrane carboxyl-terminal domain of the amyloid precursor protein that is cleaved by γ -secretase to release the amyloid- β polypeptides, which are associated with Alzheimer's disease. Nuclear magnetic resonance and electron paramagnetic resonance spectroscopy show that the extracellular amino terminus of C99 includes a surface-embedded "N-helix" followed by a short "N-loop" connecting to the transmembrane domain (TMD). The TMD is a flexibly curved α helix, making it well suited for processive cleavage by γ -secretase. Titration of C99 reveals a binding site for cholesterol, providing mechanistic insight into how cholesterol promotes amyloidogenesis. Membrane-buried GXXXG motifs (G, Gly; X, any amino acid), which have an established role in oligomerization, were also shown to play a key role in cholesterol binding. The structure and cholesterol binding properties of C99 may aid in the design of Alzheimer's therapeutics.

Alzheimer's disease (AD) currently afflicts more than 20 million people worldwide (1). The production and subsequent aggregation of amyloid- β (A β) peptides are widely thought to play a central role in most forms of AD (1, 2); accordingly, factors that increase A β production and oligomerization or that reduce its elimination increase the risk of AD. Elevated neuronal cholesterol levels increase the generation of A β (3–5), but the underlying mechanisms have yet to be elucidated.

Amyloidogenic cleavage of full-length amyloid precursor protein (APP) by β -secretase generates the transmembrane protein C99 (APP_{672–770}, also known as β -CTF, fig. S1). The transmembrane domain (TMD) of C99 is then processively but imprecisely cleaved by γ -secretase to release the A β polypeptides (6, 7). Structural information on C99 may provide insight into amyloidogenesis; however, previous structural studies have employed only low-resolution methods or have focused on TMD-containing fragments (8–12).

The backbone structure of C99 in lysomyristoylphosphatidylglycerol (LMPG) detergent micelles was determined by nuclear magnetic resonance (NMR) restraints that included residual dipolar couplings and distances derived from paramagnetic relaxation enhancement experiments (Fig. 1, figs. S2 and S3, and table S1). To prevent dimerization (11–14), we used an 800:1 LMPG-to-C99 molar ratio (see supplemental materials and methods). C99 is composed of three helical domains. A short extracellular "N-helix" (residues 688 to 694) is connected by an interfacial "N-loop" (695 to 699) to the helical TMD (700 to 723). The N-helix is embedded in the membrane surface and dynamically samples a range of orientations around the TMD helix axis (Fig. 1B). A third helix at the C terminus (residues 762 to 770) is surface-associated but is structurally uncoupled from the TMD by the intervening 38-residue "C-loop" (734 to 761). Power saturation electron paramagnetic resonance (EPR) measurements for spin-labeled C99 (Fig. 2 and fig. S4) confirm that the span of the TMD is the same in both micelles and lipid vesicles and that the N- and C-helices are surface-associated.

The NMR structure reveals that the TM helix of C99 is highly curved, with the apex of curvature being located near glycine residues 708 and 709, close to the center of the micelle (Fig. 1). Pulsed EPR double electron-electron reso-

nance (DEER) experiments confirmed that the TMD curvature also occurs in lipid bilayers (Fig. 2 and fig. S5). The measured end-to-end average distance in micelles (34.2 ± 1.1 Å) is consistent with the NMR structure and nearly identical to the distance measured in lipid vesicles (33.5 ± 1.0 Å). With the use of EPR, we found that mutation of G₇₀₈G₇₀₉ to L₇₀₈L₇₀₉ (G, Gly; L, Leu) only modestly straightens the helix (average distance now 35.3 ± 0.5 Å), suggesting that the curvature of the TMD derives only partially from the glycine pair (Fig. 2C). However, the ranges of distances sampled around the mean by the Gly⁷⁰⁸→Leu⁷⁰⁸ (G708L) and G708L/G709L mutants are dramatically reduced compared with those of the wild type (Fig. 2C). This suggests that Gly⁷⁰⁸ confers flexibility to the TMD, as predicted by molecular dynamics simulations (15). The flexibly curved nature of the TMD may be well suited for its interactions with γ -secretase. Medium-resolution electron microscopy structures of the fully assembled and activated γ -secretase (16, 17) suggest a sluice-like active site that might best accommodate a substrate with a curved TMD (see docking model in fig. S6). Analogous active sites have been observed for other intramembrane proteases (18, 19). TMD flexibility may also promote the processive cleavage of C99 by γ -secretase, with flexibility colluding with random thermal motion to allow the TMD to slide through the active-site channel. Curvature may also play a role in exposing scissile bonds for proteolytic access.

The surface-associated N-helix and N-loop immediately following the cleavage site (K687; K, Lys) for nonamyloidogenic α -secretase processing contain a number of familial AD mutation sites (20) and, in conjunction with the extracellular end of the TMD, appear to play crucial roles in determining the ratio of short versus the more toxic long forms of the A β peptides released by γ -secretase (13, 14). A space-filling surface representation (Fig. 1C) of C99 suggests that these segments may also partially occlude approach by another TMD to the glycine zipper GXXXGXXXG (X, any amino acid) sequence located on the extracellular end of the TMD, which helps to explain the weakness of C99 self-association.

The organization of the N-loop and N-helix with respect to the membrane surface and the TMD is consistent with the possibility of a lipid

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binding site centered around the N-helix/N-loop/TMD structural element, as suggested by previous studies (10, 11). Titrations of C99 with cholesterol were carried out using bicelles as the model membranes because this medium is able to solubilize cholesterol up to ~20 mole percent (mol %). Cholesterol titration of C99 results in substantial changes in NMR resonance positions for a subset of C99 peaks (Fig. 3A). The shifts in these peaks saturate at high cholesterol concentrations and can be fit by a 1:1 binding model, indicating a dissociation constant (K_d) of 5.1 ± 1.2 mol % (Fig. 3B), which falls within the range of cholesterol concentrations in mammalian plasma and organelle membranes (21), supporting the physiological relevance of this complex.

Full-length APP is likely to bind cholesterol with an avidity similar to C99 because its ectodomain is expected to have no influence on the architecture of the cholesterol binding site.

The resonances in C99 exhibiting the most profound shifts in response to cholesterol are all localized to the N-helix, N-loop, and extracellular end of the TMD and include G700, G704, and G708 (Fig. 3C). To identify specific residues crit-

ical to cholesterol binding, we employed alanine scanning mutagenesis to replace each residue in the 690 to 710 range, followed by cholesterol titrations (Fig. 3, D and E, and figs. S7 and S8). Mutations at some sites eliminated detectable cholesterol binding, even at 20 mol % cholesterol (Fig. 3E). The cholesterol binding site of C99 involves residues from the N-helix, N-loop, and TMD and is different from previously characterized cholesterol binding sites in proteins (22). Based on Fig. 3C, it is likely that N698 (N, Asn) donates a hydrogen bond to the hydroxyl head group of cholesterol, whereas E693 (E, Glu) accepts a hydrogen bond. Also essential to cholesterol binding are G700 and G704, located in the tandem GXXXG motifs of the TMD and, to a lesser degree, G708. GXXXG motifs and the related GXXXGXXXG glycine zipper sequence have long been recognized as common structural elements that can drive homo- or hetero-oligomerization of membrane proteins (23–25). Though there has been much interest in the possibility that these motifs drive homodimerization of C99 (12–15, 25), our observation that the GXXXG motifs are critical for cholesterol binding indicates an additional role for these motifs in C99. It should be added that the

Fig. 1. Structure of C99 in lysomyristoylphosphatidylcholine (LMPG) micelles. (A) Representative structure from a preliminary structural ensemble that illustrates the disorder of the N terminus and C loop and the interfacial location of the C helix. The 35 Å sphere inscribed on the structures of this figure represents a detergent micelle. (B) The 30 lowest-energy structures in which the disordered N terminus (residues 672 to 685) and cytosolic domain (726 to 770) are omitted. (C) Representative structure from (B) with atoms for glycine residues 700, 704, 708, and 709 highlighted in van der Waals mode.

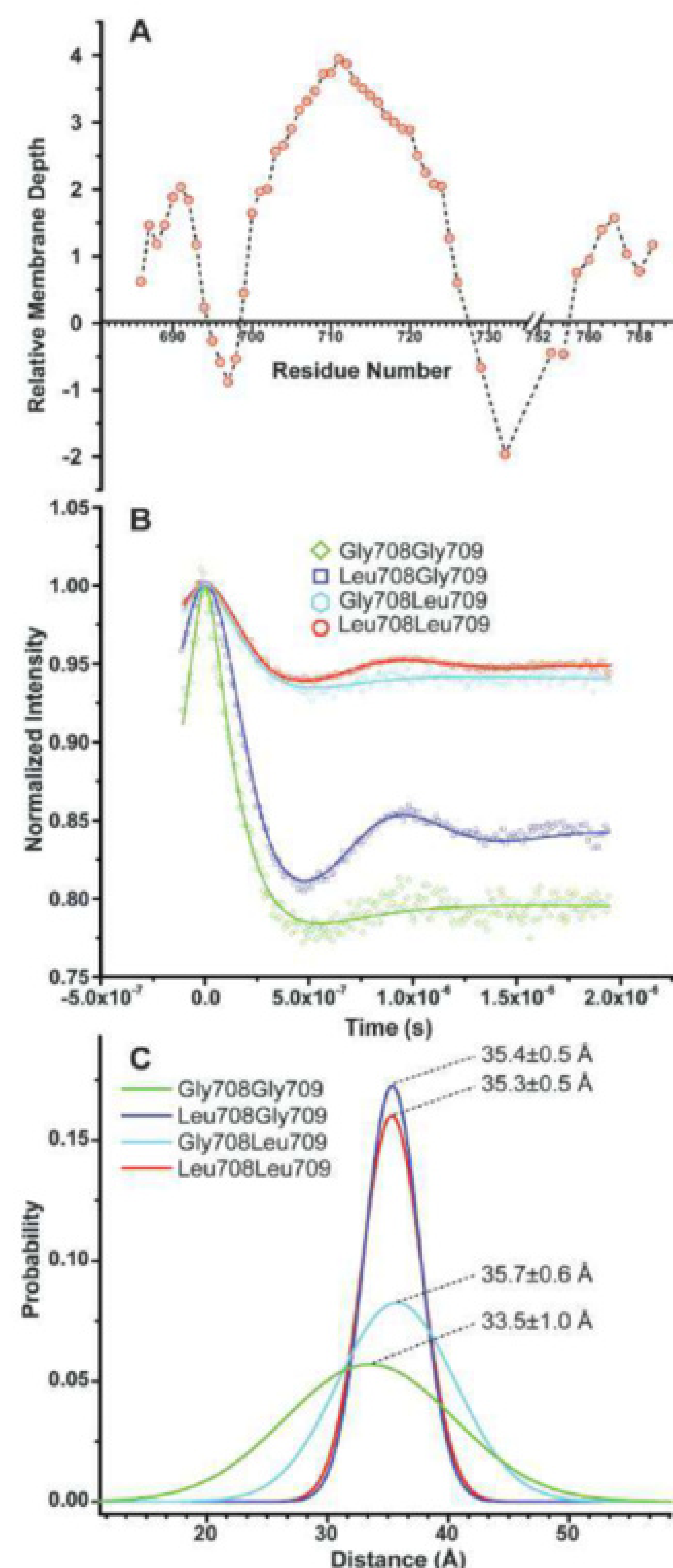
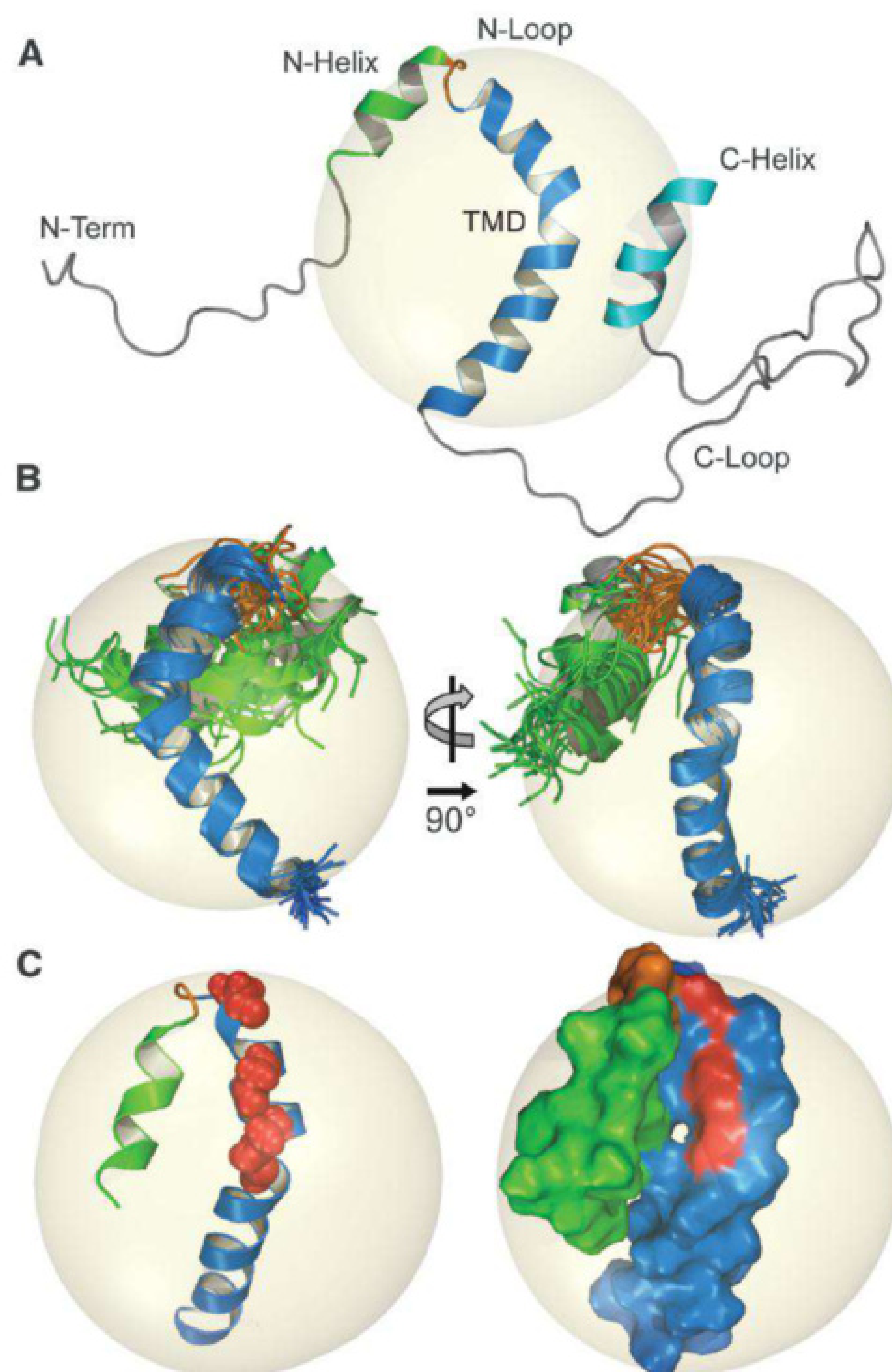


Fig. 2. EPR studies of C99 in 1:4 1-palmitoyl-2-oleoyl-phosphatidylglycerol:1-palmitoyl-2-oleoyl-phosphatidylcholine lipid vesicles. (A) Bilayer depth parameters measured for spin-labeled sites on C99 as determined from power saturation EPR measurements. Positive values indicate burial in the membrane, whereas negative values indicate exposure to water. Though the samples used for NMR structural determination contained a C-terminal purification tag (see caption to fig. S1), the samples used for measurements involving the C-terminal sites (752 to 770) did not contain a this tag, such that these measurements verify that the membrane association of the C terminus is not the result of the presence of a non-native tag sequence. (B) X-band DEER time evolutions measured at 80 K for C99 that was spin-labeled at the ends of its TMD (at sites 700 and 723). Results are shown for C99 with its wild-type (WT) sequence, except for the two spin-labeled sites, and for C99 that was additionally subjected to Gly-to-Leu mutations at G708 and G709. (C) Distance distributions between the spin labels measured from the DEER data from (B). The SD associated with each average distance relates to the uncertainty of the average, not to the population distribution around the average.

G₇₀₀AIIG₇₀₄ segment has been shown to be important in establishing the production ratio between long and short forms of the A β polypeptides by γ -secretase (13, 14) and in making that ratio susceptible to alterations by druglike small molecules known as γ -secretase modulators (26, 27).

A space-filling surface representation shows that G700 and G704 are located on the outer face

of the curved TM helix and result in that face of the helix having a locally flat surface (Fig. 1C), which is probably optimal for van der Waals interactions with cholesterol, which itself is relatively flat. Pairing the surface afforded by the GXXXG motifs with a rigid cholesterol molecule is expected to be entropically advantageous compared with association with more flexible lipids.

Binding of cholesterol to C99 appears to rely on the flexibility of the N-loop to allow induced-fit conformational changes required to optimize interactions of cholesterol with key residues in this loop and in the N-helix (fig. S9). This is supported by observation of a number of N-loop residues for which substantial changes in NMR resonance chemical shifts are observed in response to cholesterol binding [e.g., S697 (S, Ser), see Fig. 3D] but that do not appear to make direct contacts with the lipid (Fig. 3E).

The literature suggests plausible mechanisms by which complex formation between C99 or APP and cholesterol contributes to amyloidogenesis and AD. First, there are numerous reports that β - and γ -secretase associate with cholesterol-rich membrane domains often referred to as "lipid rafts" (4, 5, 28, 29). Association of C99/APP with cholesterol may favor partitioning of the protein into membrane domains enriched in the proteases of the amyloidogenic pathway. Second, cholesterol binding to C99 may play a cofactor role to promote substrate recognition or catalysis. The addition of cholesterol to purified γ -secretase in model membranes enhances the cleavage rate of purified C99 in lipid vesicles (30). Third, given that the α -secretase cleavage site (K687) is immediately adjacent to the cholesterol binding site, direct binding of cholesterol to APP could reduce nonamyloidogenic cleavage by α -secretase (31, 32). Finally, the cholesterol binding site in C99 is contained within its amyloid- β domain (C99 residues 672 to 711 correspond to A β ₄₀), such that complex formation between cholesterol and A β may contribute to the known profibrillogenic effect of membrane cholesterol (33).

In conclusion, determination of the structure of C99 and the observation that it forms an avid complex with cholesterol provide insight into amyloidogenesis. The flexibly curved TMD of C99 offers insight into how it is recognized and proteolyzed by γ -secretase, and the discovery that its GXXXG segments play critical roles in cholesterol binding reveals a previously unrecognized function for these motifs. The structure of C99 can potentially assist the design and optimization of C99-selective AD therapeutics that act by altering its interactions with γ -secretase. Moreover, development of compounds that prevent binding of cholesterol to C99 or APP may have prophylactic utility in AD.

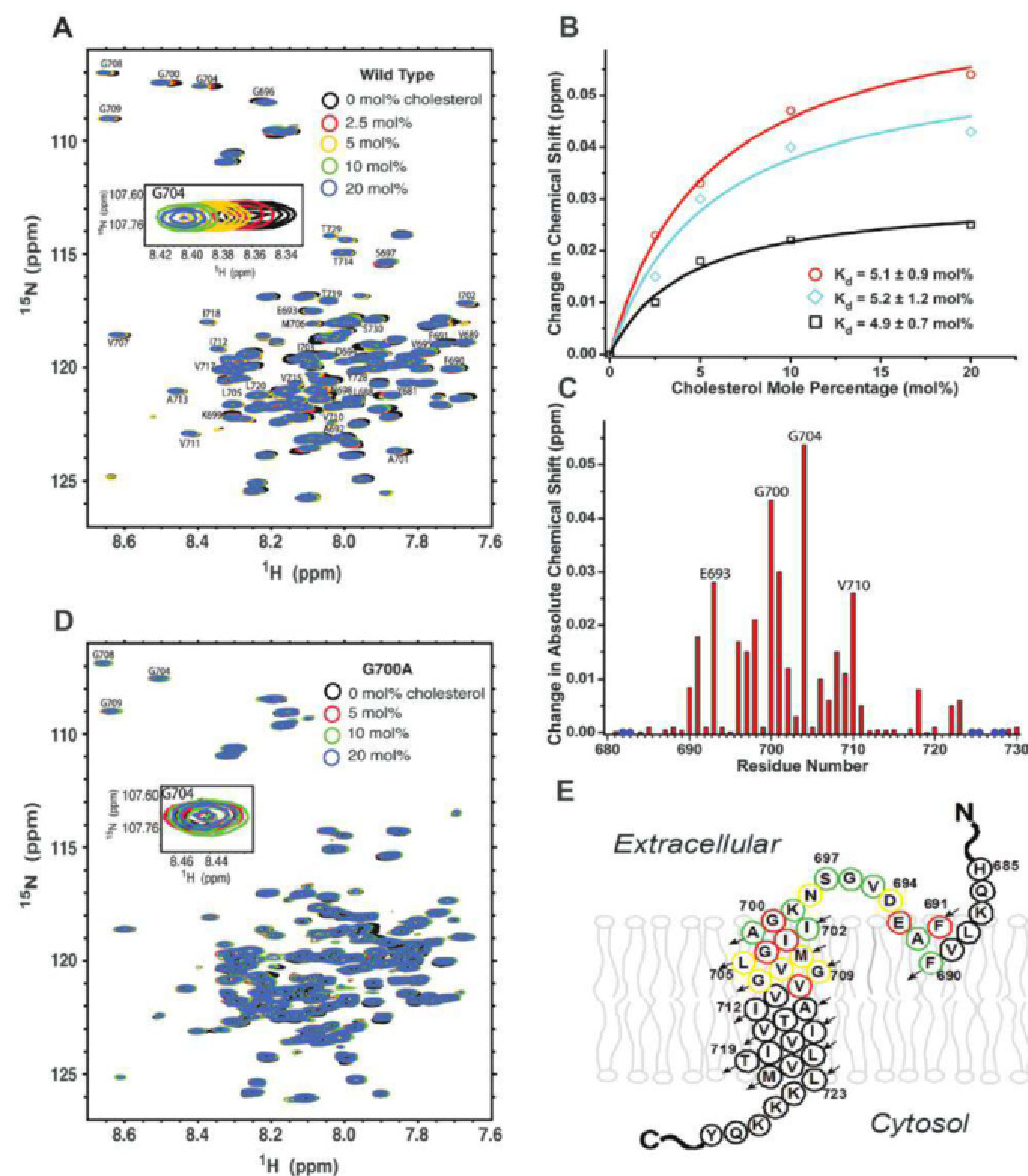


Fig. 3. Cholesterol binding to C99 in bicelles. (A) Cholesterol titration of U-¹⁵N-C99 in dihexanoylphosphatidylcholine-dimyristoylphosphatidylcholine (DHPC-DMPC) bicelles, as monitored by ¹H,¹⁵N-transverse relaxation optimized spectroscopy NMR. Cholesterol was varied from 0 to 20 mol % (relative to total moles of lipid). ppm, parts per million. T, Thr; V, Val; I, Ile; F, Phe; M, Met; D, Asp; A, Ala; Y, Tyr. (B) Changes in amide ¹H NMR chemical shifts for E693 (black), G700 (light blue), and G704 (red) in response to cholesterol titration of WT C99. Also shown are the fits of a 1:1 binding model to each data set, with resulting K_d values for complex formation indicated as well. Units of mole percent are appropriate to describe the binding of two molecules that are both associated with model membranes {mole percent = [moles cholesterol/(moles DMPC + moles cholesterol)] $\times$ 100}. (C) Changes in ¹H NMR chemical shifts for WT C99 in response to the addition of cholesterol to 20 mol % concentration. Results are shown for residues at or near the cholesterol binding site. (D) Titration of the G700A mutant form of C99 with cholesterol. (Data for other C99 mutants are shown in fig. S8). (E) Results of Ala-scanning mutagenesis. Residue color indicates the impact on cholesterol binding of substituting each position in the 690 to 710 range, as assessed by NMR. Red indicates that mutation to Ala for that site eliminates binding, yellow indicates significantly attenuated binding, and green indicates that mutation results in little change in cholesterol binding affinity. Q, Glu.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6085/1168/DC1
Materials and Methods
Figs. S1 to S9
Table S1
References (34–44)

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Computational Design of Self-Assembling Protein Nanomaterials with Atomic Level Accuracy

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We describe a general computational method for designing proteins that self-assemble to a desired symmetric architecture. Protein building blocks are docked together symmetrically to identify complementary packing arrangements, and low-energy protein-protein interfaces are then designed between the building blocks in order to drive self-assembly. We used trimeric protein building blocks to design a 24-subunit, 13-nm diameter complex with octahedral symmetry and a 12-subunit, 11-nm diameter complex with tetrahedral symmetry. The designed proteins assembled to the desired oligomeric states in solution, and the crystal structures of the complexes revealed that the resulting materials closely match the design models. The method can be used to design a wide variety of self-assembling protein nanomaterials.

Molecular self-assembly is an elegant and powerful approach to patterning matter on the atomic scale. Recent years have seen advances in the development of self-assembling biomaterials, particularly those composed of nucleic acids (1). DNA has been used to create, for example, nanoscale shapes and pat-

terns (2), molecular containers (3), and three-dimensional macroscopic crystals (4). Methods for designing self-assembling proteins have progressed more slowly, yet the functional and physical properties of proteins make them attractive as building blocks for the development of advanced functional materials (5, 6). The sophisticated protein-based molecular machines observed in natural systems—which often require self-assembly to function as, for example, cellular motors, pumps, or scaffolds—provide a suggestion of the practical potential of designed protein materials.

In any self-assembling structure, interactions between the subunits are required to drive assembly. Previous approaches to designing self-assembling proteins have satisfied this requirement in various ways, including the use of relatively simple and well-understood coiled-coil and helical bundle interactions (7–11), engineered disulfide bonds (12, 13), chemical cross-links (14), metal-mediated interactions (15, 16), templating by nonbiological materials in conjunction with

computational interface design (17), or genetic fusion of multiple protein domains or fragments that naturally self-associate (18, 19). In contrast, natural protein assemblies are most often held together by many weak, noncovalent interactions which together form large, highly complementary, low-energy protein-protein interfaces (20). Such interfaces spontaneously self-assemble and allow precise definition of the orientation of subunits relative to one another, which is critical for obtaining the desired material with high accuracy (18). Designing assemblies with these properties has been difficult because of the complexities of modeling protein structures and energetics. For instance, a pioneering study used interface design by visual inspection to design new oligomeric structures, yet the experimentally determined dimeric interfaces were largely unanticipated (21). However, recent advances (22–26), including the de novo design of a heterodimeric protein interface with atomic level accuracy (27, 28), suggest that our ability to computationally model and design protein-protein interactions is rapidly maturing.

We describe a general computational method for designing self-assembling protein materials that consists of two steps: (i) symmetrical docking of protein building blocks in a target symmetric architecture, followed by (ii) design of low-energy protein-protein interfaces between the building blocks to drive self-assembly. Here, we use as building blocks oligomeric proteins that share an element of symmetry with the target architecture. This reduces by one the number of new protein-protein interfaces that must be designed, because the interface within the oligomer contributes to the self-assembly of the subunits to the target material. Furthermore, the energetic contribution of each designed interaction is multiplied by the symmetry of the building block, which reduces the number of distinct new interactions required to overcome the entropic cost of self-assembly (21).

We used the method to design cage-like protein nanomaterials with either tetrahedral (T) or octahedral (O) point group symmetry (Fig. 1). An

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assembly with symmetry T requires 12 copies of a protein molecule arranged in 12 symmetry-related orientations, whereas symmetry O requires 24 molecules. Both point groups can be generated from sets of three-fold rotational symmetry axes (Fig. 1A), which allows the use of protein trimers with C3 symmetry as building blocks; in each case, only a single new interface between the trimeric building blocks is required for self-assembly. In this study, 271 naturally trimeric protein structures (29) were docked symmetrically in both the T and O target architectures by aligning the three-fold axis of each building block with the three-fold axes in the target architecture and then systematically sampling the two remaining rigid body degrees of freedom, radial displacement and axial rotation, in increments of 1 Å and 1°, respectively (Fig. 1, B and C). For each docked configuration in which no clashes between the backbone and beta carbon atoms of adjacent building blocks were present, a simple proxy for interface size and complementarity was computed to gauge the “designability” of the configuration (Fig. 1, C and D) (29). Around each of the 10 (O) or 20 (T) most designable configurations for each building block, a set of input structures for design was generated by sampling the radial displacement and axial rotation of the subunits more finely (0.1 Å, 0.5°). For each of these input structures, symmetric RosettaDesign calculations (30, 31) were used to design a new amino acid sequence for the protein that resulted in low-energy, symmetric protein-protein interactions between the trimeric building blocks (Fig. 1, E and F). Designs with the lowest predicted binding energies and geometrically complementary interfaces of sufficient size were further optimized using RosettaDesign and interactive design in Foldit (32). Eight T and 33 O designs derived from 15 distinct natural trimeric proteins, containing on average nine mutations per monomer, were selected for experimental characterization (table S1).

Genes encoding the designed proteins and the corresponding wild-type trimers were constructed and cloned into an expression vector that appended an 11-residue peptide substrate for fluorescent modification by the *Escherichia coli* acyl-carrier protein synthase AcpS (33). *E. coli* cells expressing the proteins were lysed, the proteins were fluorescently labeled in the clarified lysates by the addition of AcpS and the CoA-488 fluorophore, and the apparent size of each protein was visualized by subjecting the labeled lysates to polyacrylamide gel electrophoresis (PAGE) under nondenaturing (native) conditions. Out of 7 T and 17 O designs that expressed solubly (table S1), one designed protein of each architecture revealed a shift in apparent size relative to the corresponding wild-type trimer that suggested self-assembly to the desired material (Fig. 2A). Size-exclusion chromatography (SEC) of the labeled lysates confirmed the change in apparent molecular weight for the two designs (fig. S1). Genes encoding the octahedral design (“O3-33”;

nine mutations from the wild-type protein), the tetrahedral design (“T3-08”; eight mutations), and the corresponding wild-type trimeric proteins were then subcloned into an expression vector that appended C-terminal (His)₆ tags, after which the proteins were expressed and purified by nickel-affinity chromatography and SEC.

The designed protein O3-33 eluted from the SEC column as a single peak with an apparent size of about 24 subunits (Fig. 2B). The wild-type protein from which O3-33 was derived [Protein Data Bank (PDB) ID 3N79] did not assemble to a higher-order structure; it eluted from the column mostly as trimers, with a small peak corresponding to a dimer of trimers (Fig. 2C). Analytical ultracentrifugation revealed that the designed protein sedimented as a single discrete species with a Stokes radius of 7.3 nm, in close agreement with the radius of the designed 24-subunit assembly (fig. S2). A point mutation (Ala167Arg) that introduced unfavorable steric clashes at the designed interface disrupted the material, which suggests that the observed self-assembly is due to the designed

interface (Fig. 2D). Negative-stain electron microscopy (EM) of O3-33 revealed fields of monodisperse particles of the expected size (~13 nm), many of which strikingly resembled projections of the design model along its two-fold, three-fold, or four-fold symmetry axes (Fig. 3A). A single-particle reconstruction of O3-33 obtained by EM analysis under cryogenic conditions clearly recapitulated the architecture of the design model, which verified that the protein assembles in solution as designed (Fig. 3C and fig. S3).

We solved crystal structures of O3-33 to evaluate the accuracy of our design protocol at high resolution. Structures from two different crystal forms confirmed that the designed material adopts the target architecture and that the designed interface is responsible for driving self-assembly; the higher-resolution (2.35 Å) crystal form is shown in Fig. 3. The structure proved remarkably similar to the design model: The backbone root mean square deviation (RMSD) over all 24 chains is 1.07 Å and is lower if calculated by using only the residues at the interface (0.85 Å). The high resolution of the structure allowed

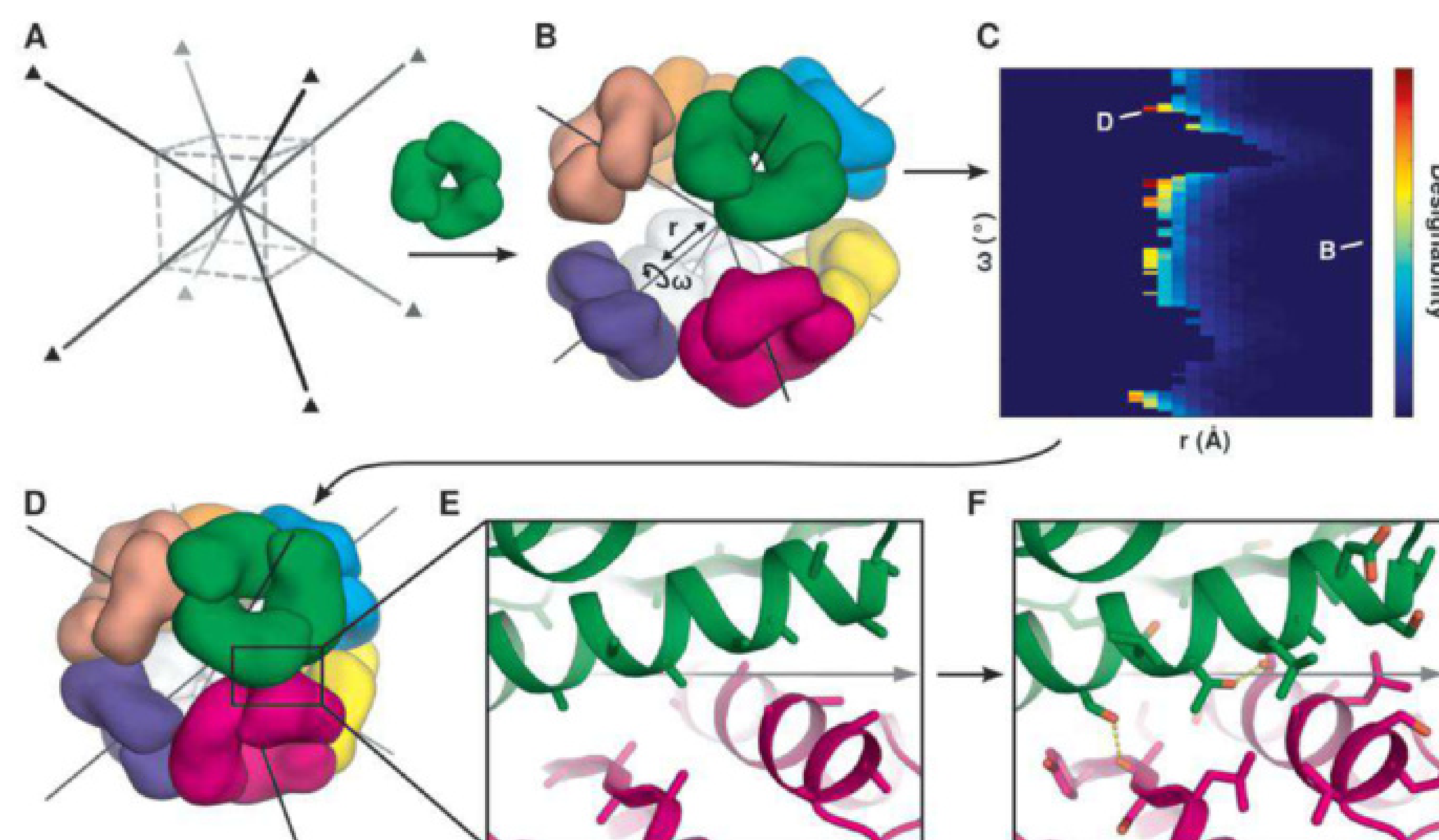


Fig. 1. General approach to designing self-assembling protein nanomaterials. (A) First, a target symmetric architecture is chosen. Octahedral point group symmetry is used in this example; the three-fold rotational axes are marked here by triangles and shown as black lines throughout. The dashed cube is shown to orient the viewer. A symmetric oligomer which shares an element of symmetry with the target architecture, here a C3 symmetric trimer (green), is selected as a building block. (B) Multiple copies of the building block are symmetrically arranged in the target architecture by aligning their shared symmetry axes. The preexisting organization of the oligomeric building block fixes several (in this case four) rigid-body degrees of freedom (DOFs). The two remaining DOFs, radial displacement (r) and axial rotation (ω), are indicated. (C) Symmetrical docking is performed by systematically varying the two DOFs (moves are applied symmetrically to all subunits) and computing the suitability of each configuration for interface design (red: more suitable; blue: less suitable). Points corresponding to the docked configurations in (B), in which the building blocks are not in contact, and (D), a highly complementary interface, are indicated. (E) Closer view of the interface in (D). The interface lies on an octahedral two-fold symmetry axis shown as a gray line. In all steps before interface design, only backbone (shown in cartoon) and carbon beta (shown in sticks) atoms are considered. (F) Sequence design calculations are used to create low-energy protein-protein interfaces that drive self-assembly of the desired material. Designed hydrogen bonds across the interface are indicated by dashed lines.

confident determination of the side-chain configurations at the designed interface, which revealed that the atomic contacts closely match those in

the design model (Fig. 3E). The asymmetric unit of the designed interface consists of one alpha helix packing against a beta strand, a loop, and

Fig. 2. Experimental characterization of O3-33, T3-08, and T3-10. (A) Native PAGE of fluorescently labeled (from left) 3n79-wt, O3-33, 3ftt-wt, and T3-08 in lysates. Bands corresponding to the designed octahedral (O3-33) and tetrahedral (T3-08) assemblies are indicated with asterisks. SEC chromatograms of nickel-purified (B) O3-33, (C) 3n79-wt, (D) O3-33(Ala167Arg), (E) T3-08, (F) T3-10, (G) 3ftt-wt, and (H) T3-08(Ala52Gln) collectively demonstrate that the assembly of the designed proteins is a result of the designed interfaces.

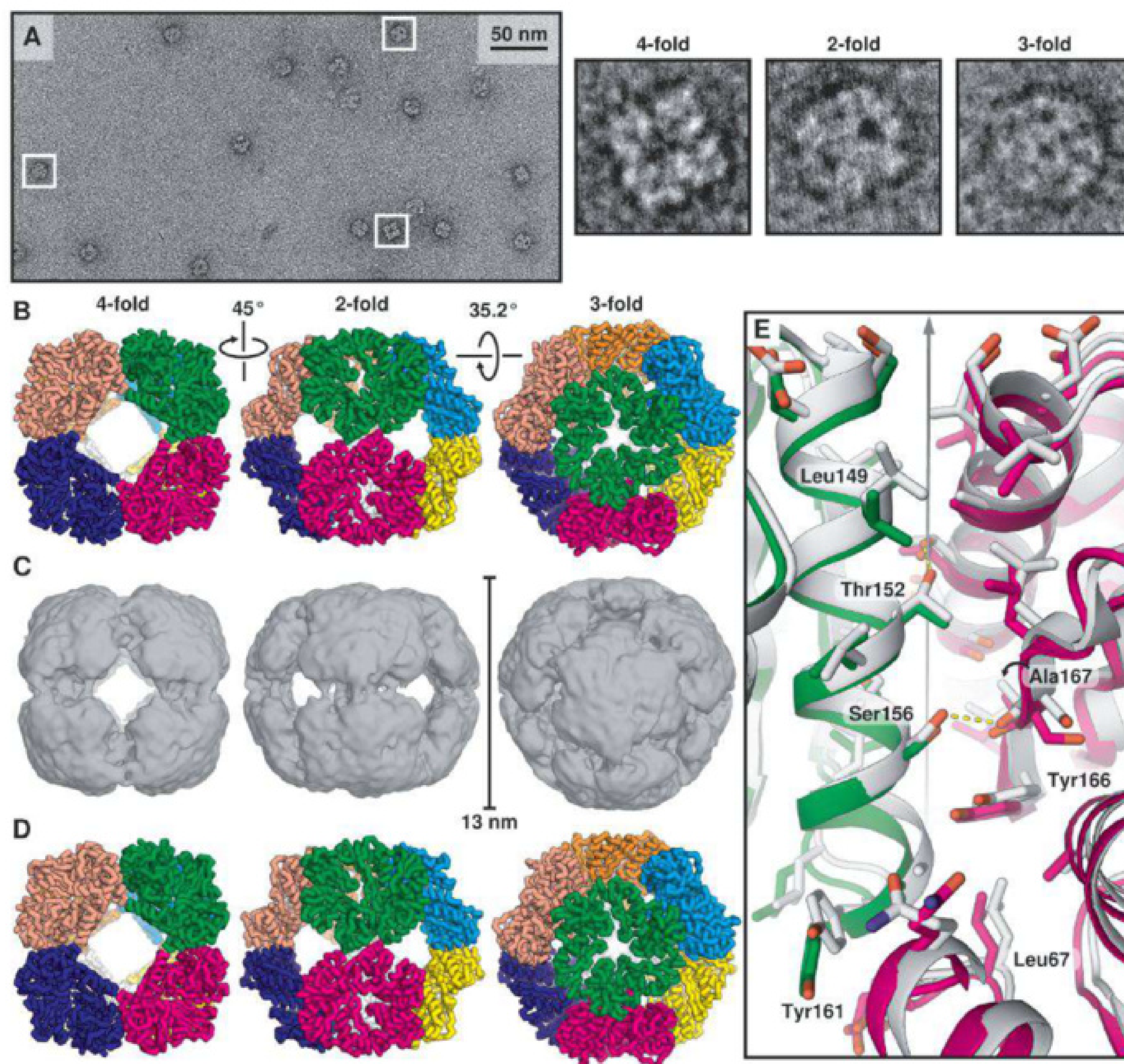
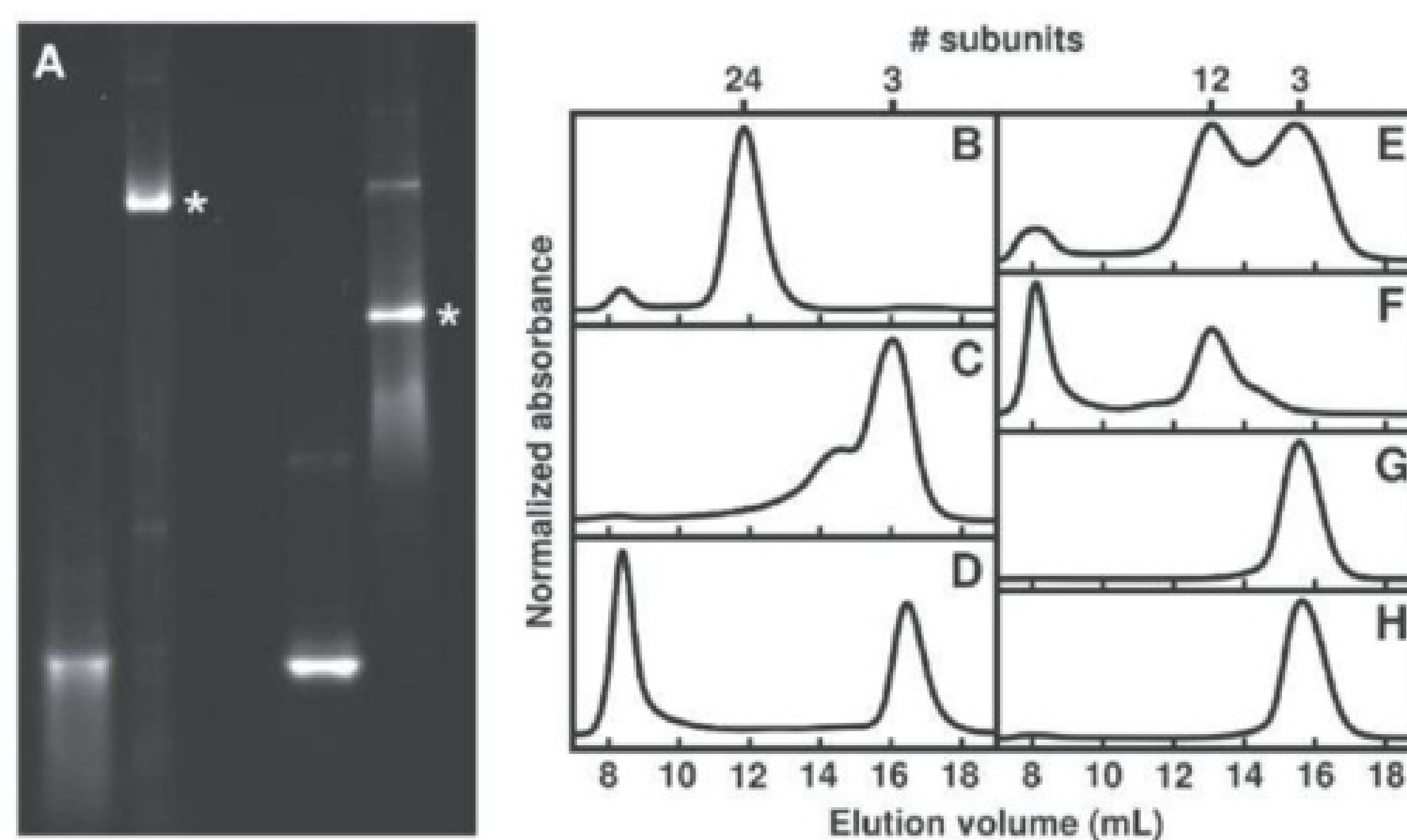


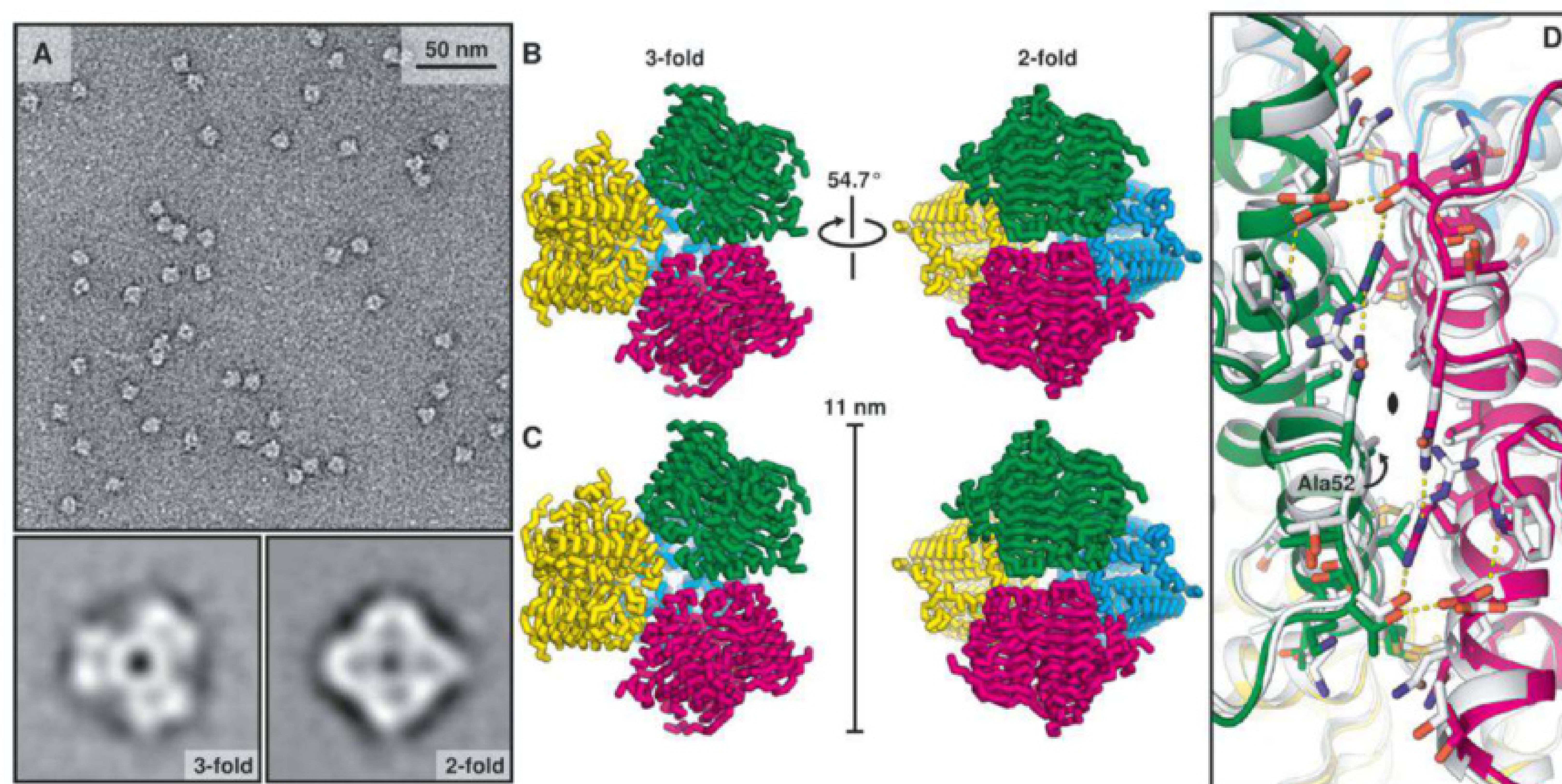
Fig. 3. Structural characterization of O3-33. (A) A representative negative-stain electron micrograph of O3-33. Selected particles (boxed in white) that resemble views of the design model along its four-fold, two-fold, and three-fold rotational axes, shown in (B), are enlarged at right. (B) The O3-33 design model, depicted in ribbon format. Each trimeric building block is shown in a different color. (C) The density map from a 20 Å resolution cryo-EM reconstruction of O3-33 clearly recapitulates the architecture of the design model. (D) The crystal structure of O3-33 (R32 crystal form). Images in (B) to (D) are shown to scale along the three types of symmetry axes present in point group O. (E) The designed interface in O3-33, highlighting the close agreement between the crystal structure (green and magenta) and the design model (white). Oxygen atoms are red; nitrogens, blue. Hydrogen bonds between the building blocks are shown as yellow dashes, and an octahedral two-fold rotational axis that passes through the interface is shown as a gray line. Residues in which substitution disrupted self-assembly (see fig. S4) are labeled.

the symmetrically related helix in a neighboring building block. Several ordered water molecules were resolved at the designed interface that contribute bridging hydrogen-bonding interactions between neighboring building blocks. Truncation of designed interface residues to alanine disrupted octahedral self-assembly (fig. S4). For example, the Ser156Ala mutation, which alters O3-33 by the removal of only two atoms out of 2827 total atoms in the subunit, significantly impaired assembly. This result underscores the importance of both the detailed atomic contacts designed by our protocol and the multiplicative effect of the symmetry of the system: The Ser156Ala mutation results in the loss of 24 interface hydrogen bonds in the fully assembled material.

The designed protein T3-08 appeared by SEC to be in a slow equilibrium between two states comprising 3 and ~12 subunits (Fig. 2E). The corresponding wild-type trimeric protein (PDB ID 3FTT) eluted from the column as trimer only (Fig. 2G). Disruption of the designed interface by a point mutation, Ala52Gln, again suggested that the designed interface is responsible for the observed self-assembly (Fig. 2H). A crystal structure of T3-08 revealed that the protein assembles to the desired tetrahedral architecture, but the trimeric building blocks are slightly rotated about the shared trimeric-tetrahedral three-fold rotational axes, which subtly alters the atomic contacts at the designed interface relative to the design model and results in a backbone RMSD of 2.66 Å over all 12 subunits (fig. S5).

We designed two additional variants of T3-08 (table S1) to determine whether we could preferentially stabilize the designed configuration over the unanticipated configuration observed in the T3-08 crystal structure. One of the variants, T3-10, which contained three mutations relative to T3-08 intended to provide better hydrophobic packing near the tetrahedral three-fold interface (fig. S6), was purified by nickel-affinity chromatography and appeared by SEC to self-assemble efficiently to the tetrahedral state, yielding little detectable trimer (Fig. 2F). Negative-stain EM images of T3-10 revealed monodisperse particles of the expected size (~11 nm), averages of which closely resembled projections of the design model along its two-fold and three-fold symmetry axes (Fig. 4, A and B). A crystal structure of T3-10 verified that the original designed configuration was stabilized as intended; the backbone RMSD between the T3-10 crystal structure and the T3-08–T3-10 design models is 0.62 Å (Fig. 4, B and C). As observed for O3-33, the atomic contacts at the designed interface, which consists of two alpha helices and two short loops, closely match those in the design model (Fig. 4D). This result illustrates how small alterations to the protein sequence at the designed interface may allow fine control over the structure of the resulting material.

Fig. 4. Structural characterization of T3-10. (A) A representative negative-stain electron micrograph of T3-10. At bottom, averages of the particles resemble views of the design model along its two-fold and three-fold rotational axes, shown in (B). (B) Backbone representation T3-08–T3-10 design model, depicted as in Fig. 3B. (C) The T3-10 crystal structure. Images in (B) and (C) are shown to scale along the two types of symmetry axes present in point group T. (D) The designed interface in T3-10, revealing the close agreement of the crystal structure (green and magenta) to the design model (white). A network of polar interactions observed in the crystal structure at the designed interface is indicated by yellow dashes. The interface is viewed along an indicated tetrahedral two-fold rotational axis. Alanine 52 is labeled; when mutated to glutamine in T3-08, it disrupts assembly of the designed material.



Our results establish a method by which self-assembling protein materials may be designed with high accuracy. The design strategy, combining symmetrical docking with interface design, is conceptually simple and generally applicable to the design of a broad range of symmetric materials. In addition to the finite, cage-like materials described here, unbounded materials in one, two, or three dimensions [i.e., fibers (helices), layers, or crystals] may be designed by choosing an appropriate target symmetric architecture. Although, in the present study, we used naturally occurring oligomeric proteins as building blocks, novel oligomeric building blocks could first be designed from monomers and, after structural validation, used in the design of higher-order assemblies with the attendant advantages of hierarchical assembly, or, with improvements in our symmetrical docking protocol, larger self-assembling systems could be designed directly from monomeric building blocks. The atomic-level accuracy of our designed materials demonstrates that using designed protein-protein interfaces to drive self-assembly results in highly ordered materials with superior rigidity and monodispersity. With further development, designed self-assembling protein materials similar to those described here could form the basis of advanced functional materials and custom-designed molecular machines with wide-ranging applications.

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Supplementary Materials

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Generic Indicators for Loss of Resilience Before a Tipping Point Leading to Population Collapse

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Theory predicts that the approach of catastrophic thresholds in natural systems (e.g., ecosystems, the climate) may result in an increasingly slow recovery from small perturbations, a phenomenon called critical slowing down. We used replicate laboratory populations of the budding yeast *Saccharomyces cerevisiae* for direct observation of critical slowing down before population collapse. We mapped the bifurcation diagram experimentally and found that the populations became more vulnerable to disturbance closer to the tipping point. Fluctuations of population density increased in size and duration near the tipping point, in agreement with the theory. Our results suggest that indicators of critical slowing down can provide advance warning of catastrophic thresholds and loss of resilience in a variety of dynamical systems.

Natural populations can experience catastrophic collapse in response to small changes in environmental conditions, and recovery after such a collapse can be exceedingly difficult (1, 2). Tipping points marking population collapse and other catastrophic thresholds in natural systems may correspond to

a fold bifurcation in the dynamics of the system (3–6). Even before crossing a tipping point, a system may become increasingly vulnerable to perturbations due to loss of “ecological resilience” (i.e., size of the basin of attraction) (4, 7). There has been a growing interest in the possibility of using generic statistical indicators, primarily based on critical slowing down, as early warning signals of impending tipping points in various systems (8–16). In dynamical systems theory, critical slowing down refers to the slow recovery from small perturbations in the vicinity of bifurcations (8, 17). As the system approaches a bifurcation, the time needed to recover from

perturbations becomes longer (11, 18) and hence the system becomes more correlated with its past, leading to an increase in autocorrelation. In addition, the perturbations accumulate and result in an increase in the size of the fluctuations (10). Other statistical indicators, such as skewness, have also been proposed as warning signals because of the change in stability landscape before bifurcations (19).

An increase in variance or autocorrelation of fluctuations of the system has been observed to precede a regime shift in a lake ecosystem (13), abrupt climate change (9, 14), transitions in coordinated biological motion (20), and the cascading failure of the North America Western Interconnection power system in 1996 (21); these findings suggest the existence of bifurcation-type tipping points and associated critical dynamics in many systems. Because the complex dynamics underlying these systems makes it difficult to determine the nature of the transitions, studies in controlled systems are required. Recent studies in laboratory water fleas (12) and cyanobacterial monoculture (16) measured the warning signals under controlled conditions. However, the transition in the deteriorating-environment experiment of water fleas, probably due to a transcritical bifurcation, was noncatastrophic (fig. S1). Moreover, in both systems the tipping points were not determined directly by experiments. Thus, neither study constituted a demonstration of early warning signals before an experimentally mapped fold bifurcation in a live system. Such a study can also test directly the possibility of using critical slowing down to indicate loss of ecological

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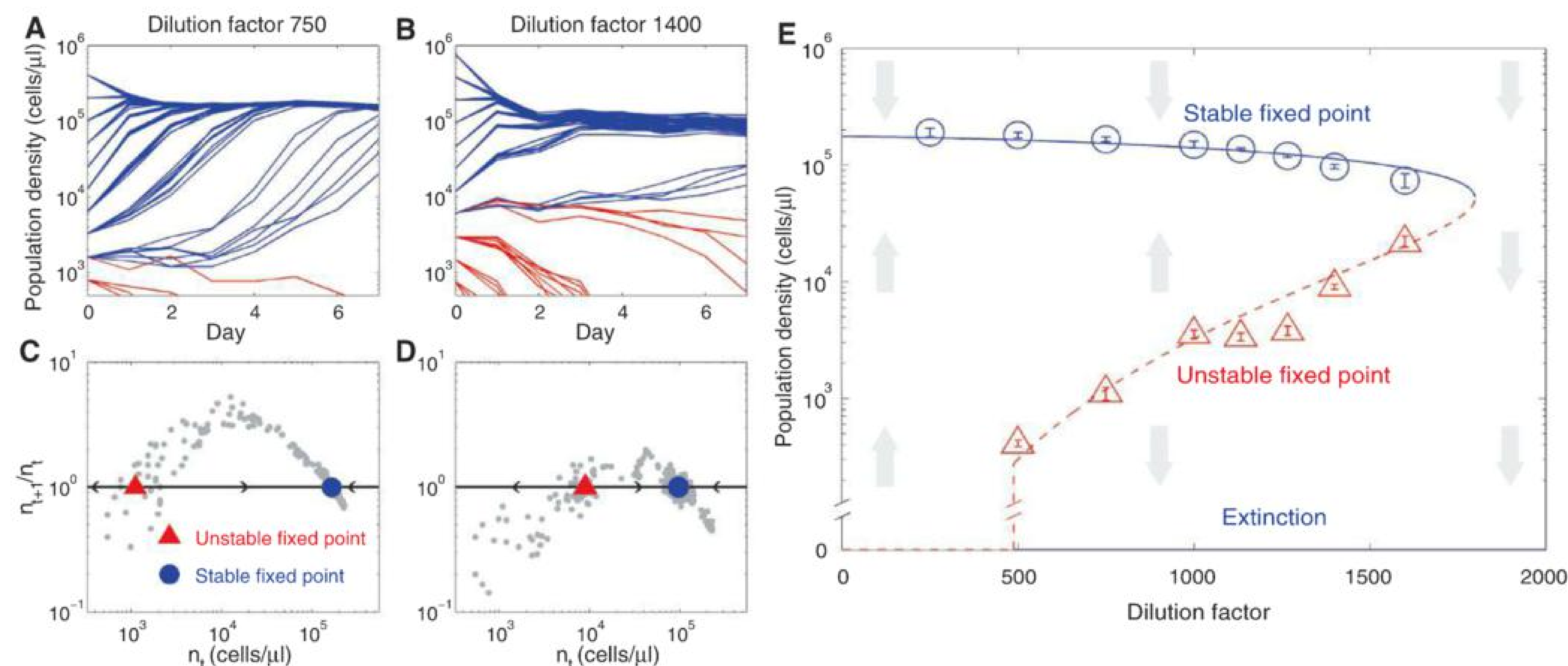


Fig. 1. Cooperative growth of yeast in sucrose leads to bistability and a fold bifurcation. (A to D) Individual populations started at different initial densities were grown in 2% sucrose with daily dilutions into fresh media [(A) and (C), dilution factor 750; (B) and (D), dilution factor 1400]. Small populations below a critical density (an unstable fixed point) went extinct (red traces), whereas larger populations converged (blue traces) and maintained a stable density (a stable fixed point). As shown in (C) and (D), the stable and unstable fixed points

can be identified as fixed points at which the ratio of population densities between subsequent days $n_{t+1}/n_t = 1$, where n_t is the population density at day t ($t = 1$ to 6). As the daily dilution factor is increased, the two fixed points approach each other. (E) The stable and unstable fixed points measured by experiments are shown as symbols (fig. S3); the lines represent a typical fit given by the two-phase growth model (fig. S4). A fold bifurcation occurs at a dilution factor where the stable and unstable fixed points “collide” and annihilate.

resilience, as suggested previously in models (11). Here, we present an experimentally constructed bifurcation diagram and clear evidence of both loss of resilience and critical slowing down before a fold bifurcation leading to population collapse.

A catastrophic threshold may underlie the collapse of many natural populations subjected to a deteriorating environment. Likely examples are the collapse of sardine stocks off California and Japan in the late 1940s and the collapse of the Canadian cod fishery in the 1990s (1, 2). One reason for such sudden collapse is that the per capita growth rate of many populations is maximal at intermediate densities and negative at low densities—a phenomenon called the strong Allee effect (22). At high population densities, the per capita growth rate is reduced because of resource competition; at low population densities, the per capita growth rate can be negative because of difficulties in finding mates, forming groups for hunting or predator avoidance, or engaging in other cooperative behaviors. The Allee effect has been observed across many species and has major impacts on the dynamics and viability of populations (23, 24). As the environment deteriorates, a population subject to a strong Allee effect is pushed across a fold bifurcation, beyond which it enters a catastrophic collapse that can be difficult to reverse. Near the bifurcation, a population becomes less resilient because the basin of attraction around the stable state shrinks, elevating the

chance of extinction by stochastic perturbations. The approach of catastrophic thresholds and the accompanying loss of resilience can occur without a substantial preceding drop in population density; they are also difficult to predict because of incomplete understanding of the population dynamics and the lack of field data to determine model parameters (25). Thus, finding early warning signals before the catastrophic collapse of a given population has important implications for successful environmental management (4, 26).

We used the cooperative growth of budding yeast in sucrose to realize the Allee effect. Yeast cells hydrolyze sucrose outside of the cell, hence yeast can benefit from the hydrolysis products of other cells in the population (27). This cooperative breakdown of sucrose makes the per capita growth rate of yeast ~40% higher at intermediate cell densities than at low cell densities (fig. S2), thus displaying the desired Allee effect.

We observed bistability in population density by starting yeast cultures with a wide range of initial cell densities and performing daily di-

lutions into fresh media. During the daily dilution, only a small fraction (e.g., 1 in 750, for dilution factor 750) of the population was transferred to the new media with 2% sucrose. This is equivalent to introducing a mortality rate and led to a negative growth rate at low initial cell densities. Therefore, cultures starting below a critical density went extinct, whereas cultures starting at higher initial densities survived and reached a finite stable fixed point (Fig. 1, A and B). As a result of stochastic perturbations, we found that replicate populations starting near the critical density (unstable fixed point) split, with some populations surviving and others going extinct. An analysis of the change in population density between subsequent days as a function of the density on the first day allowed for the identification of both stable and unstable fixed points for a given dilution factor (Fig. 1, C and D). As expected, the cooperative growth of yeast populations in sucrose led to maximal growth at intermediate densities.

Performing these experiments for eight different daily dilution factors (28), we mapped out

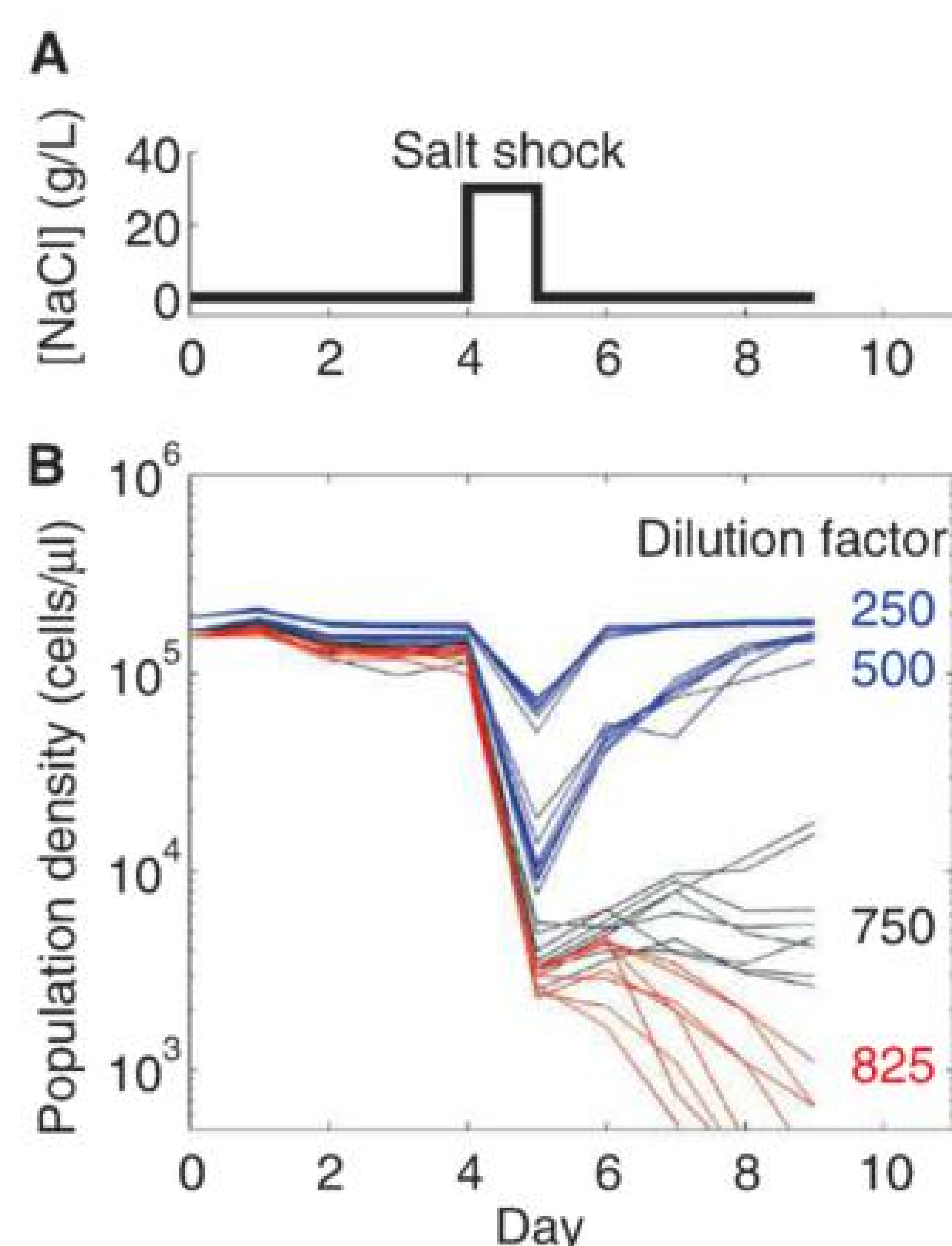


Fig. 2. The resilience of yeast populations decreases with dilution factor. The populations were grown at fixed daily dilution factors and stabilized during the initial 4 days. (A) Sodium chloride (30 g/liter) produced a salt shock during day 5. (B) Populations at low dilution factors were able to recover; those at higher dilution factors were pushed across the unstable fixed point by the perturbation and went extinct.

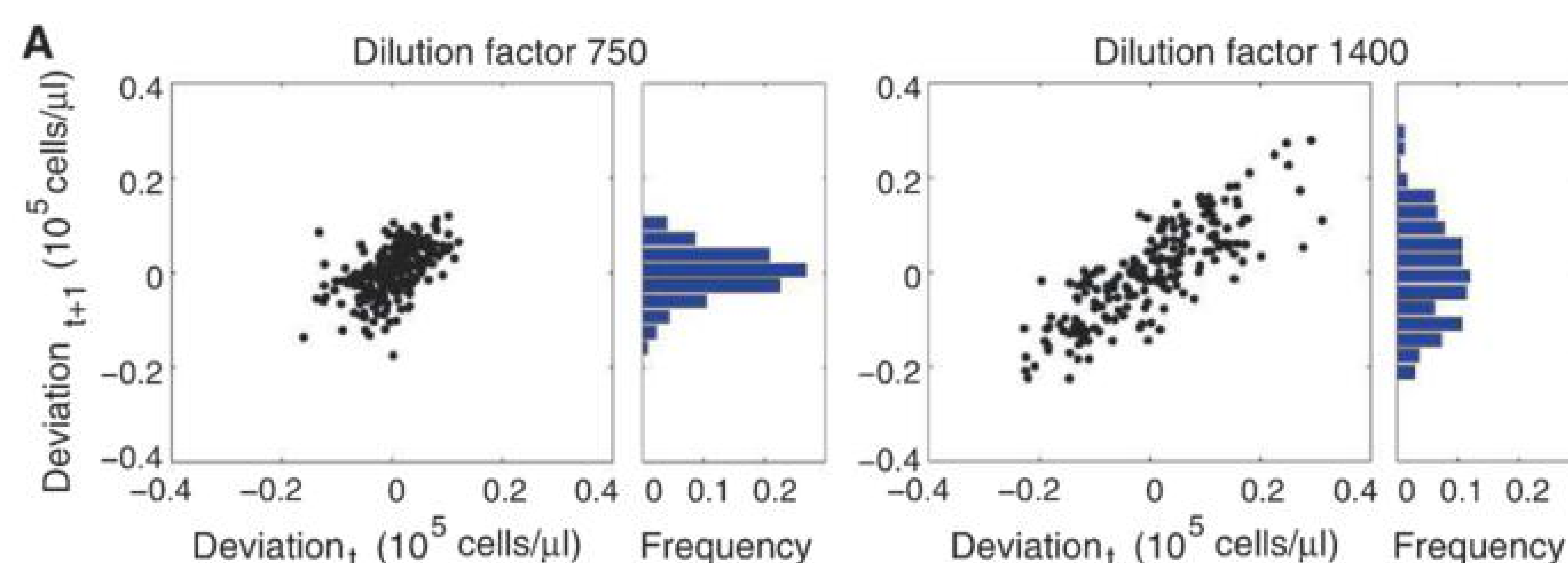


Fig. 3. Critical slowing down leads to increasing variation and autocorrelation time in population density near the fold bifurcation. (A) Changes in the distribution and temporal correlation of deviations from the stable fixed point. One condition (dilution factor 750) is far from the bifurcation; another condition (dilution factor 1400) is closer to the bifurcation. (B to E) Plots of dilution factor versus coefficient of variation (B), standard deviation (C), autocorrelation time (D), and skewness (E). Both the size and the time scale of the fluctuations of the population density increased before the fold bifurcation; no systematic change in skewness was observed. Indicators were calculated on the basis of an ensemble of at least 46 replicate populations over a span of 5 days after they converged to the stable fixed point. Deviation was calculated by subtracting the sample mean on each day. Data shown in the histogram include 5 days; $t = 1$ to 4 for temporal correlation plots. Error bars are SEs given by bootstrap (28).

an experimental bifurcation diagram describing yeast growth in sucrose (Fig. 1E). At small dilution factors (250), yeast populations were always able to reach a stable nonzero fixed point. At intermediate dilution factors (500 to 1600), the population displayed bistability due to cooperative growth, with one stable fixed point at a finite population density and the other at extinction. As the dilution factor (i.e., the mortality rate) was increased, the population density at the nonzero stable fixed point decreased and the population density at the unstable fixed point increased (Fig. 1, C to E). A fold bifurcation occurred where the stable and unstable fixed points “collide” and annihilate. For higher dilution factors, populations always collapse, as extinction is the only stable state. We constructed a simple deterministic model of yeast growth based on the experimentally observed slow exponential growth at low population densities and faster logistic growth at higher population densities (figs. S2 and S4). With this simple two-phase growth model, we were able to fit the experimental bifurcation diagram using parameter values that agree well with those measured in independent experiments (table S1).

Closer to the bifurcation, we expect the population to become more vulnerable to perturbations (4, 11). In our experimental bifurcation diagram, loss of ecological resilience is manifested as the shrinking basin of attraction between the stable and the unstable fixed points (Fig. 1E). Natural disturbances are then more likely to push the population across the unstable fixed point, leading to extinction. We tested the resilience of yeast populations at different dilution factors by a salt shock of sodium chloride for 1 day (Fig. 2). As expected, populations at low dilution factors were able to recover from the perturbation, whereas those at higher dilution factors were not able to withstand the shock and went extinct. The decreasing capacity of a population to recover from perturbations without shifting to an alternative state provides an empirical demonstration of loss of resilience before a tipping point.

We then used our experimental system to test whether theoretically predicted early warning signals can be observed before the fold bifurcation. Once the populations at different dilution factors reached equilibrium (stable fixed points), we tracked the fluctuations of population density for 5 days and calculated the proposed early warning signals over an ensemble of at least 46 replicate yeast populations (28). Upon approach of the fold bifurcation, we observed a clear increase in the size of the fluctuations (Fig. 3, A to C). Both the coefficient of variation and the standard deviation began to increase substantially around dilution factor 1000, well before the bifurcation. The coefficient of variation increased by more than a factor of 4; the standard deviation more than doubled.

We also measured the autocorrelation time, which characterizes the typical time scale of

correlation between fluctuations at different points in time. The autocorrelation time increased markedly for dilution factors larger than 1200, from less than 2 days to more than a week (Fig. 3, A and D). In addition, we estimated the characteristic return time to the stable fixed point by fitting an exponential relaxation (fig. S5). We observed a clear increase in return time, which in theory equals the autocorrelation time (11, 18).

The observation that fluctuations of population density became larger and more correlated clearly indicated critical slowing down. Moreover, our experimental observations were supported by simulations of a stochastic difference equation based on the two-phase model of yeast growth (fig. S6). Previous studies in different systems, including a whole-ecosystem experiment in lakes (13), laboratory cyanobacterial monoculture (16), and paleoclimate data (9, 14), revealed early warning indicators before a fold bifurcation predicted by models. Our study with an experimentally constructed fold bifurcation revealed all the indicators associated with critical slowing down. The observation of early warning signals in various systems lends support to the notion that critical slowing down is a universal phenomenon preceding transitions in complex systems.

A primary advantage of laboratory experiments is that they enable comparison of the quality of different early warning signals in a well-controlled system. With this goal in mind, we tested skewness, a suggested early warning signal not based on critical slowing down (19). The skewness, which measures the asymmetry of fluctuations in population density, is expected to increase in magnitude near the fold bifurcation because of asymmetrical changes in the stability landscape. Indeed, there has been some experimental support for the change in skewness as an early warning signal (12, 13). However, in our data there was no systematic change in skewness (Fig. 3E). Stochastic simulations of yeast growth suggest that no change in skewness should be detectable with our sample size (fig. S7). Moreover, as the dilution factor is increased in the model, both sides of the stability landscape become flatter and the magnitude of skewness actually decreases, in contrast to previous theoretical arguments (19). Thus, both the experimental data and simulation suggest that skewness is not a good warning signal in our system.

Our results show that critical slowing down can provide early warning signals for loss of resilience before a fold bifurcation that leads to catastrophic population collapse. Ecosystem management depends on monitoring and maintaining resilience, because loss of resilience renders ecosystems more vulnerable to undesirable shifts (4, 7, 11, 29, 30). Our results and work by others (12, 13, 16) suggest that a set of generic indicators may aid in the sustainable management of fragile ecosystems. Signals of critical slowing down based on time series demand ob-

servations over a long span, which are often difficult to obtain in the field; therefore, if other indicators based on spatial structure can be identified, they could be complementary to the early warning signals studied here.

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Supplementary Materials

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B Cell Receptor Signal Transduction in the GC Is Short-Circuited by High Phosphatase Activity

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Germinal centers (GCs) generate memory B and plasma cells, which are essential for long-lived humoral immunity. GC B cells with high-affinity B cell receptors (BCRs) are selectively expanded. To enable this selection, BCRs of such cells are thought to signal differently from those with lower affinity. We show that, surprisingly, most proliferating GC B cells did not demonstrate active BCR signaling. Rather, spontaneous and induced signaling was limited by increased phosphatase activity. Accordingly, both SH2 domain-containing phosphatase-1 (SHP-1) and SH2 domain-containing inositol 5 phosphatase were hyperphosphorylated in GC cells and remained colocalized with BCRs after ligation. Furthermore, SHP-1 was required for GC maintenance. Intriguingly, GC B cells in the cell-cycle G₂ period regained responsiveness to BCR stimulation. These data have implications for how higher-affinity B cells are selected in the GC.

T cell-dependent immune responses result in the selection of high-affinity B cells, a process that occurs in the germinal center (GC) and depends on high-rate somatic mutation of V regions to generate variants. Resultant GC B cells can differentiate into either memory or plasma cells, which confer lasting humoral immunity (1). During this process, the B cell receptor (BCR)

promotes the selective survival or expansion of higher-affinity GC cells, but how this occurs is unclear. It is possible that BCRs on higher-affinity GC B cells transduce a stronger, more sustained or qualitatively different signal. A second possibility is that higher-affinity BCRs more effectively capture antigens (Ag), which are subsequently presented to helper T cells, resulting in higher-affinity B cells obtaining more T cell-derived survival or proliferative signals (1, 2). Though BCR function is central to the process of GC B cell selection, BCR signaling in the GC is not well understood.

In vivo BCR signaling in GC B cells is of great interest, as these cells are activated and undergo continuous selection based on BCR affinity. Such study is complicated by the fact that

GC B cells are rare, transient, and heterogeneous. Furthermore, some GC B cells express an immunoglobulin G-containing BCR, which mediates different signaling than the immunoglobulin M (IgM) BCR (3–5). Heterogeneity may also confer distinct signaling phenotypes on GC BCRs, which would be obscured in experiments using assays of populations rather than single cells.

To overcome these issues, we have used an IgM BCR B1-8 transgenic (Tg) mouse (6, 7). The Tg encodes a germline Vh186.2 rearrangement that is common in the anti-nitrophenyl (anti-NP) response when combined with the 2 to 3% of Tg B cells expressing Vλ1. Such B cells in the Tg mice mount a vigorous GC response to NP-chicken gamma globulin (CGG) immunization (6, 7). These B cells undergo mutation in the Vλ1 chain, but because of the dominant role of the heavy chain in determining affinity, light-chain mutations have very limited effects on the affinity (6). Thus, these mice represent a source of large numbers of relatively homogeneous GC B cells expressing only IgM, thus obviating differential signaling by isotype-switched BCRs (3); moreover, the unimmunized Tg mice contain the exact naïve precursors of these GC B cells.

We first examined B cell signaling in freshly isolated splenic Ag-specific (i.e., Vλ1⁺) GC (peanut agglutinin, PNA⁺) and non-GC (PNA[−]) cells that were immediately fixed, followed by flow cytometric analysis of phosphorylated proteins (fig. S1, A and B) (8). In immunized mice, non-GC cells are mainly nonresponding bystander cells and serve as an internal control, used in addition to naïve splenocytes. Naïve cells demonstrated

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Fig. 1. Spontaneous and ligand-induced BCR signaling in GC, non-GC, and resting B cells. **(A)** BCR-linked basal signaling in gated populations of GC, non-GC, and naïve B cells from instantly fixed total splenocytes harvested from day-13 NP-CGG immunized mice (fig. S1). Fixed cells were treated with or without calf intestinal phosphatase (CIP) and then labeled with antibodies specific for phosphorylated proteins. **(A)** Histograms show representative results of tyrosine phosphorylation from CIP-treated (red) and untreated (blue) cells, overlaid for direct comparison. The y axes show relative cell numbers. Bar charts show net median fluorescence intensity (MFI) indicating basal phosphorylation calculated by subtracting MFI of CIP-treated from CIP-untreated cells. Error bars denote SEM from at least five independent experiments, each using cells pooled from spleens of at least two mice for each group. **P* < 0.05 and ***P* < 0.01 MFI of GC or non-GC compared to naïve cells. **(B)** Response of GC, non-GC, and resting B cells to BCR ligation. Total splenocytes from day-13 NP-CGG immunized mice were stimulated ex vivo with anti-μ (15 μg/ml) for 5 min. Levels of Syk, Blnk, Tyr, p38, and Erk phosphorylation in gated GC and non-GC cells were measured. Profiles of GC-unstimulated (red filled area), GC anti-IgM-stimulated (red open trace), non-GC-unstimulated (blue filled area), and non-GC anti-IgM-stimulated (blue open trace) cells are overlaid for direct comparison.

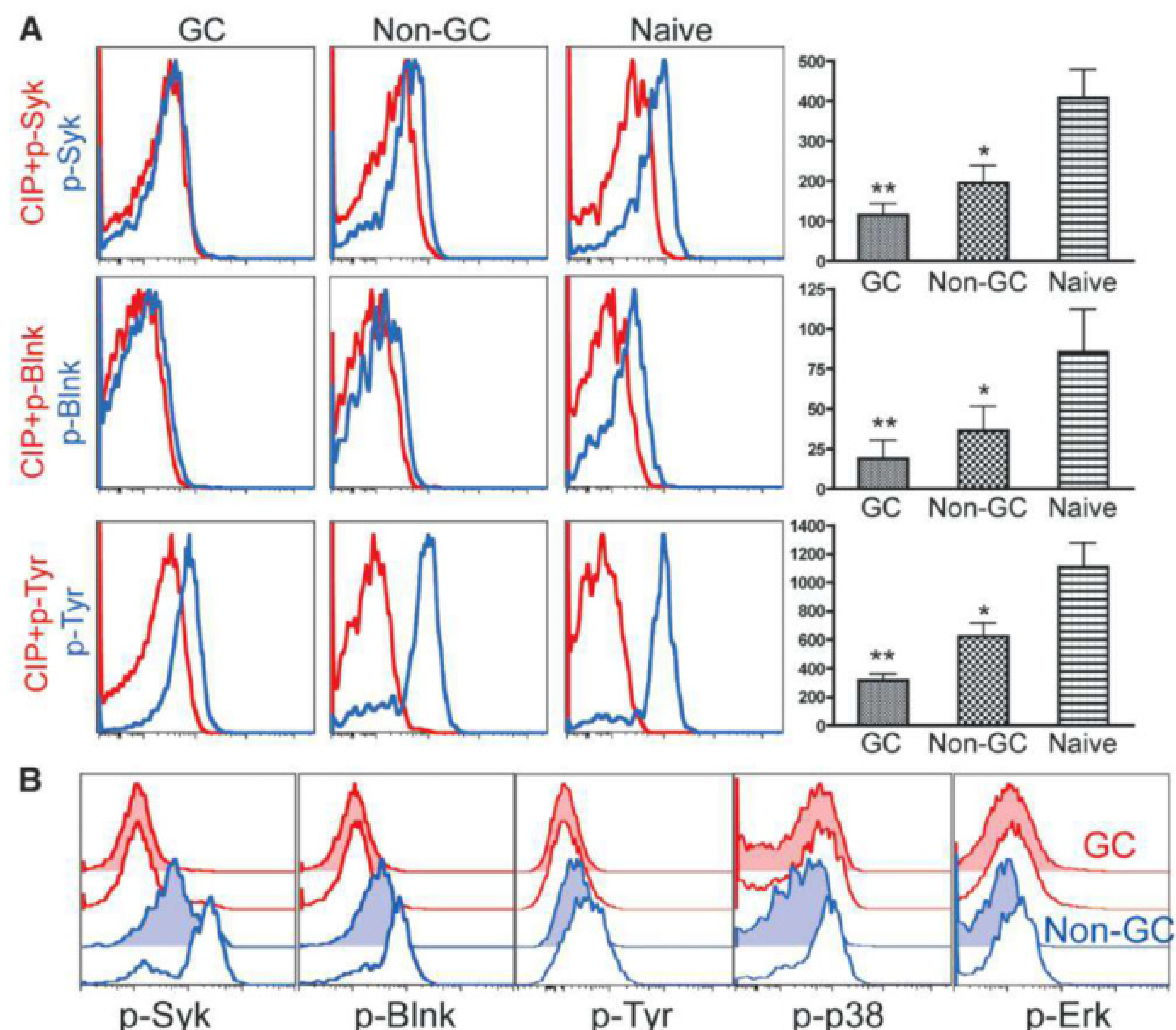


Fig. 2. Analysis of phosphatase-dependent regulation of BCR signaling in GC cells. **(A)** Total splenocytes from day-13 NP-CGG immunized mice were stimulated with 5 mM (green) or 10 mM (blue) H_2O_2 , followed by detection of p-Syk and p-Blk by flow cytometry in gated GC and non-GC Ag-specific cells. These panels are representative of three independent trials each of three mice. **(B)** Assessment of interaction between BCR ligation and phosphatase inhibition. Total splenocytes from immunized mice as in (A) were stimulated with 15 μ g/ml anti-IgM (red), 5 mM H_2O_2 (blue), or both (green), and generation of p-Syk was assessed by flow cytometry. Panels are representative of three or more experiments. **(C)** Indo1AM-loaded total splenocytes from immunized mice were stimulated with 15 μ g/ml anti-IgM (red), 5 mM H_2O_2 (blue), or both (green). Stimuli were added after acquiring events for 5 min to establish a basal level. Profiles of stimulants in gated GC (top) and non-GC (bottom) populations are overlaid. The y axis shows the indo1 violet-to-blue fluorescence ratio, an indicator of intracellular Ca^{2+} levels. **(D)** Compiled responses of GC and non-GC cells treated as in (C). Background-subtracted MFI was calculated from gates drawn before (180-s time, background) and after (200-s time, beginning at approximately the initial peak of response to anti- μ) stimulation. Error bars show mean + SEM of net MFI from four independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **(E)** Ca^{2+} flux to ionomycin, used as a positive control indicating equal responsiveness of GC and non-GC cells.

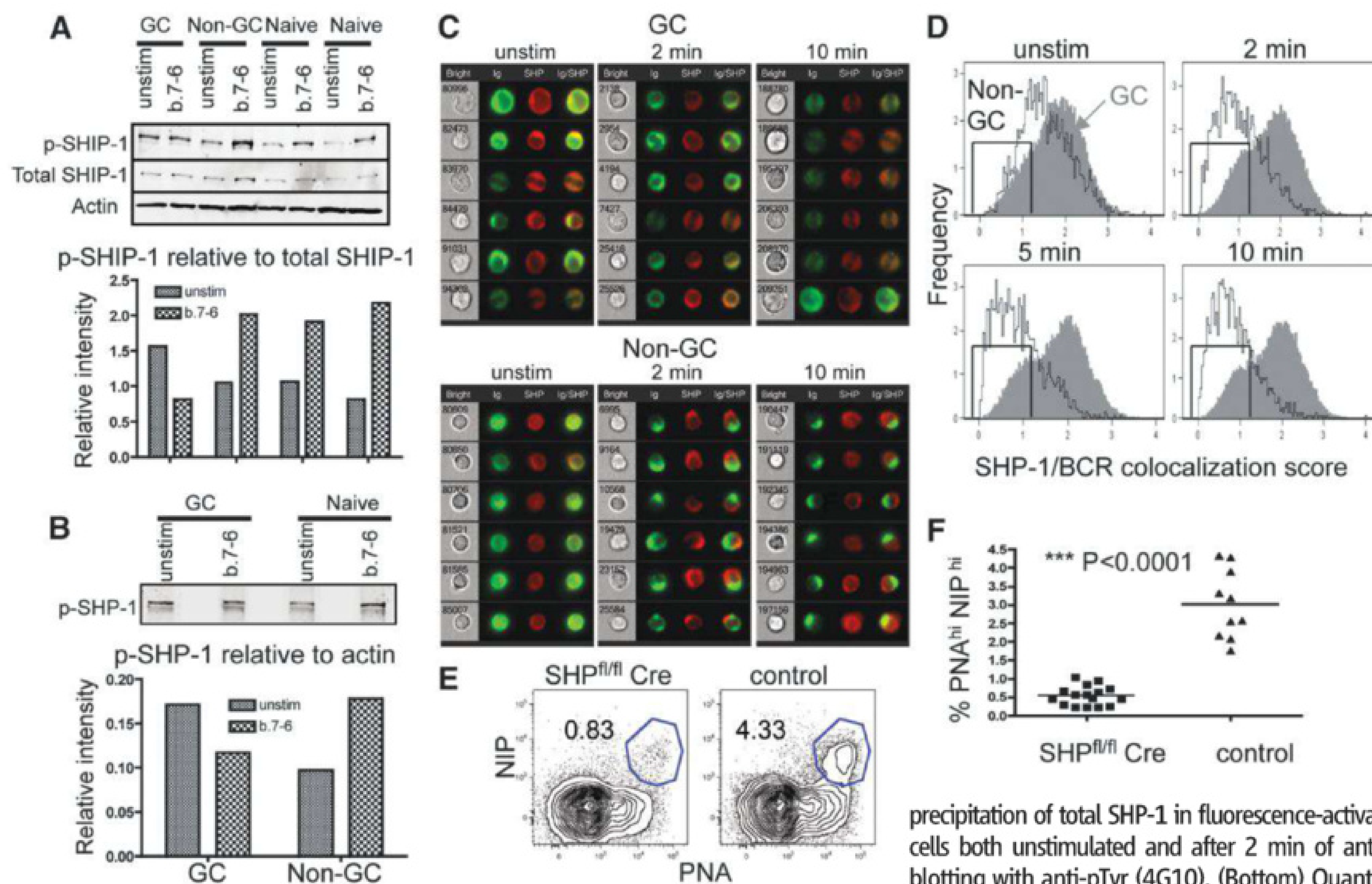
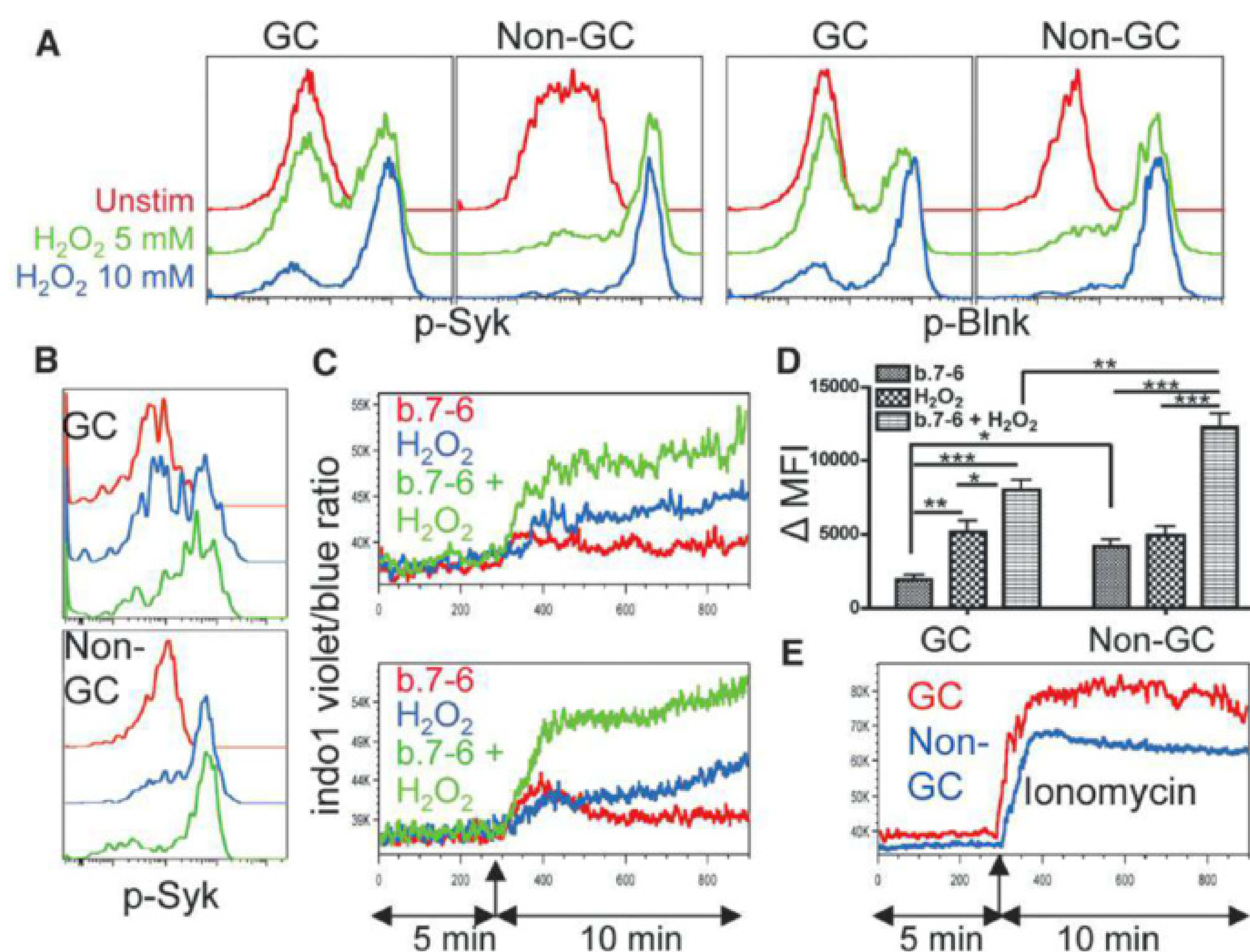


Fig. 3. Differences in phosphorylation and localization of SHIP-1 and SHP-1 in GC and naïve B cells after and without BCR ligation. **(A)** (Top) SHIP-1 phosphorylation (Tyr¹⁰²⁰) was determined by Western blot of lysates from unstimulated and anti-IgM stimulated (5 min) fluorescence-activated cell-sorted GC, non-GC, and naïve B cells. β -actin was used as a loading control. (Bottom) Quantitation of blot shown in top is MFI of gated p-SHIP-1 bands relative to total SHIP-1. Blots shown are representative of three similar experiments. **(B)** (Top) Tyrosine phosphorylation of SHP-1 was determined by immunoprecipitation of total SHP-1 in fluorescence-activated cell-sorted GC and naïve B cells both unstimulated and after 2 min of anti-IgM stimulation, followed by blotting with anti-pTyr (4G10). (Bottom) Quantitation of blots shown in top is MFI of gated p-SHP-1 relative to β -actin, which was determined by Western

blot on parallel samples of the same lysates. Data representative of four similar experiments. **(C and D)** High-throughput imaging cytometric analysis of SHIP-1/BCR association. Total splenocytes were either left unstimulated or stimulated with anti-IgM (b.7-6) for 2, 5, 10, 15, and 30 min (see fig. S7A for 15- and 30-min summary data). **(C)** Representative images of GC (top row) and non-GC (bottom row) B cells captured by the Amnis Imagestream X (Amnis, Seattle, Washington). **(D)** Colocalization of SHIP-1 and BCR was measured in gated non-GC ($\lambda_1^+PNA^{lo}$) and GC ($\lambda_1^+PNA^{hi}$) as similarity scores in GC (shaded) and non-GC (open) B cells at multiple time points with respect to BCR ligation (all time points summarized in fig. S7A). The box depicts a gate drawn at <1.2 similarity, which was used to calculate the percentage of GC and non-GC cells demonstrating substantial SHIP-1/BCR dissociation at various times after BCR ligation (fig. S7B). **(E and F)** Effect of deletion of SHP-1 in B cells on the ongoing GC response. The strategy for tamoxifen-induced deletion using a new B cell-specific inducible Cre enzyme (hCD20-TamCre) and SHP-1^{fl/fl} mice is detailed in fig. S8. SHP-1^{fl/fl} mice with or without (control) the Cre Tg were immunized with NP-CGG, treated from days 9 to 12 with tamoxifen, and then analyzed at day 14. **(E)** Representative flow cytometric analysis of splenocytes from experimental (left) and control (right) mice, detecting Ag-specific GC cells as PNA⁺/NIP⁺ among gated B220⁺ B cells. Numbers are percentages of B cells in the gate. **(F)** Data from three independent experiments showing loss of GC B cells (B220⁺PNA⁺NIP⁺) upon SHP-1 deletion in B cells.

basal tyrosine phosphorylation of the tyrosine kinase Syk (p-Syk, Tyr³⁵²) and its substrate BLNK (p-BLNK, Tyr⁸⁴) (Fig. 1A), both of which are proximal signal transducer elements of the BCR. Results were consistent with genetic and inhibitor studies (9, 10). GC B cells, however, had little detectable p-Syk or p-BLNK and much reduced total phosphotyrosine (p-Tyr) compared with either non-GC or naïve Ag-specific B cells (Fig. 1A). In contrast, p-p38 (Thr¹⁸⁰/Tyr¹⁸²), p-ribosomal S6 (Ser²³⁵/Ser²³⁶), and p-Akt (Thr³⁰⁸), were present in GC cells at similar or higher levels compared to control cells (fig. S1C).

The lack of BCR signaling could be explained by low in vivo Ag exposure or inherent resistance to BCR signals. To distinguish these possibilities, we stimulated splenocytes from day 13 postimmunization (fig. S2A) with the monoclonal antibody to IgM (anti-IgM), b.7-6 (Fig. 1B), F(ab')₂ anti-IgM, or NP-bovine serum albumin (fig. S2, B and C). In contrast to non-GC cells, GC B cells

demonstrated little if any induction of several phosphoproteins downstream of the BCR, suggesting that they were inherently antigen-refractory.

To evaluate BCR down-regulation, we stimulated GC B cells directly with fluorescently labeled anti-IgM, which allowed us to electronically gate the analysis on cells with equivalent surface Ig levels (fig. S3A). Such GC cells again showed little induction of phosphoproteins compared with the non-GC cells with equivalent BCR expression. At 15 min poststimulation, GC B cells still did not contain elevated levels of p-Syk, excluding kinetic differences (fig. S3B). More than 97% of the cells were still viable at the end of stimulatory cultures (fig. S4A); consistent with this, GC cells were not inert—they generated p-Erk and p-p38 in response to phorbol 12-myristate 13-acetate (PMA)/ionomycin stimulation, which bypasses the BCR (fig. S2D). Furthermore, resistance to BCR-mediated generation of p-Syk was not a general property of activated B cells (fig. S4B).

To assess more proximal BCR signaling events, we evaluated basal phosphorylation of CD79, which is part of the BCR complex. GC cells had about half the amount of total CD79 compared with resting B cells (fig. S5, A to C) as expected, because GC cells have less surface Ig (fig. S3A). Upon stimulation with anti-IgM, only non-GC and naïve B cells showed an increase in the amount of p-CD79, revealing a defect at the earliest measurable events of BCR signaling (fig. S5, A to C).

Heightened phosphatase activity could explain the failure of GC B cells to accumulate increased tyrosine-phosphorylated CD79, Syk, and BLNK (11, 12); hence, we examined effects of Tyr phosphatase inhibition with H₂O₂. Exposure of GC B cells to H₂O₂ (fig. S6A) resulted in increased phosphorylation of Syk in non-GC cells, as reported by Wienands *et al.* (10). p-Erk, which is presumably downstream of Syk activation, was similarly affected, as were total p-Tyr levels. Notably, H₂O₂ rescued substantial p-Syk, p-Erk, and

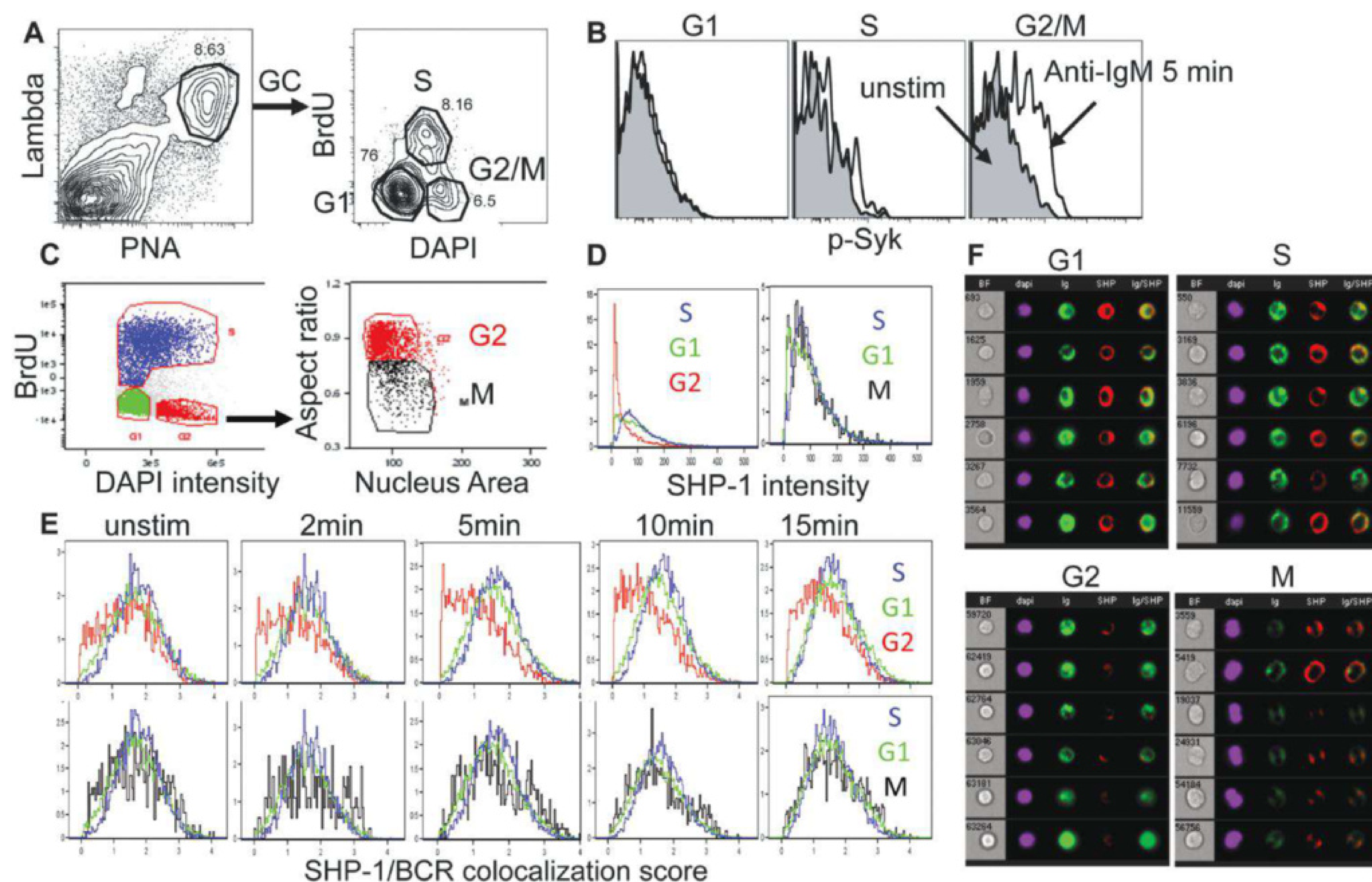


Fig. 4. BCR signal transduction in GC B cells at the G₂/M phase of the cell cycle. Mice at day 13 postimmunization with NP-CGG were injected intravenously once with 3 mg of 5-bromo-2'-deoxyuridine (BrdU) and sacrificed 1 hour later. Splenocytes were isolated and either treated with anti-IgM or not for 5', then fixed and stained as described in the supplemental materials and methods. (A) Flow cytometry gating to identify GC B cells (left) and then separate them into cell-cycle compartments based on 4',6-diamidino-2-phenylindole (DAPI) and BrdU staining (right). (B) Phosphorylation of Syk in response to BCR ligation was measured in gated populations based on (A) and as labeled: G₁ (left), S (center), and G₂/M (right). Histograms of p-Syk staining in unstimulated cultures (gray-filled) and stimulated cultures

(open) are shown. Data are representative of four independent mice from two independent experiments, all with similar results. (C to F) GC cells were analyzed on the Imagestream X for SHP-1 intensity and SHP-1/BCR colocalization during different phases of the cell cycle. GC B cells were prepared and treated as in (A) but analyzed on the Imagestream after staining as in Fig. 3. (C) DAPI and BrdU identify G₁ (green), S (blue), and G₂/M phases (red/black). G₂ and M phases were separated based on nuclear area and aspect ratio. (D) Analysis of total SHP-1 expression as a function of cell cycle. (E and F) Analysis of SHP-1/BCR colocalization as a function of cell cycle during the G₂ phase compared with the G₁ and S phases. Data are representative of two experiments.

p-Tyr expression in GC cells. Phosphorylation was somewhat higher in the non-GC cells, however, particularly at early time points. These data suggest that established phosphatase activity restrains spontaneous phosphorylation in GC B cells.

Consistent with higher Tyr-phosphatase activity, maximal phosphorylation in the GC required a higher concentration of H_2O_2 compared with non-GC B cells (Fig. 2A). To determine if H_2O_2 directly disinhibited BCR signaling, we pretreated splenocytes of immunized animals with a suboptimal concentration of H_2O_2 before anti-IgM stimulation. Such pretreatment synergistically enhanced BCR-induced Syk phosphorylation compared with anti-IgM or H_2O_2 alone (Fig. 2B). Treatment with anti-IgM and H_2O_2 (Fig. 2, C and D) also synergistically elicited more Ca^{2+} flux in both GC and non-GC cells compared with either anti-IgM or H_2O_2 alone; anti-IgM alone stimulated little Ca^{2+} flux in GC cells. The response to ionomycin showed that GC cells are at least as equipped to flux Ca^{2+} as non-GC cells (Fig. 2E). These results indicate that phosphatase activity limits the ability of GC B cells to carry out Tyr phosphorylation and Ca^{2+} flux in response to BCR stimulation.

To assess this, we measured both Tyr and Ser/Thr phosphatase activity in lysates from purified GC, non-GC, and naïve B cells. As predicted, there was more Tyr and Ser/Thr phosphatase activity in the GC than in naïve cells (fig. S6B). The finding of increased Ser/Thr phosphatase activity may be connected to impaired GC B cell generation of p-Erk and pp38 in response to BCR ligation (Fig. 1B). Although this could have been due to a block in proximal BCR signaling, there was also less Erk and p38 phosphorylation evident upon PMA/ionomycin stimulation in GC B cells (fig. S2D), consistent with increased Ser/Thr phosphatase activity.

We next examined whether SH2 domain-containing phosphatase-1 (SHP-1), a Tyr phosphatase known to regulate BCR signaling upon BCR ligation (13–15), may be responsible for reduced GC BCR signaling. Possible SHP-1 substrates include CD79, Syk, Vav, BLNK, and CD22 (16). Furthermore, we investigated SH2 domain-containing inositol 5 phosphatase (SHIP-1), which also regulates BCR signaling (17, 18), as well as Src family members because they can exert negative effects on BCR signaling (19). The activity of these proteins is increased by phosphorylation (20, 21). We found more p-SHIP-1 (Tyr¹⁰²⁰), p-SHP-1, and p-Src (Tyr⁴¹⁶) in unstimulated GC compared with non-GC and naïve B cells (Fig. 3, A and B, and fig. S6C). Ex vivo stimulation with anti-IgM increased phosphorylation of SHP-1, SHIP-1, and Src proteins (most likely Lyn) in non-GC and naïve B cells, reflecting normal regulation of signaling (15, 19, 20). However, p-SHIP-1, p-SHP-1, and p-Src did not rise in anti-IgM-stimulated GC B cells; in fact, they consistently decreased upon BCR ligation.

SHP-1 (15) and SHIP-1 (22) are constitutively associated with the BCR in resting cells. Unstimulated GC B cells demonstrated more

SHP-1/BCR (Fig. 3, C and D, and fig. S7, A and B) and SHIP-1/BCR (fig. S7C) colocalization than did non-GC cells. BCR ligation resulted in brisk dissociation of both SHP-1 and SHIP-1 from BCR in non-GC cells. In these cells, BCR and SHP-1 relocated to opposite poles of the cell, consistent with biochemical analysis of resting B cells (15). This dissociation was sustained for SHP-1 but transient for SHIP-1. In contrast, GC B cells barely showed any dissociation upon BCR ligation, though BCR/SHP-1 colocalization was reduced in a minor subset, albeit with slower onset and less sustained duration than in non-GC cells (Fig. 3D, fig. S7B, and see below). The differential localization of phosphatases in GC versus non-GC cells is further consistent with the regulation of BCR signaling in GC B cells by phosphatases.

To determine the importance of SHP-1 in B cells during GC responses, we crossed SHP-1^{fl/fl} (23) with a new Tg strain (hCD20.TamCre) that allows B cell-specific inducible Cre action (fig. S8, A to C). B cell SHP-1 expression was reduced by injecting tamoxifen at days 9 to 12 after immunization (fig. S8, A to C), which resulted in marked reduction of GC B cell frequencies (Fig. 3, E and F), indicating that SHP-1 and, presumably, regulation of BCR signaling is required for maintaining the GC reaction.

If GC BCRs do not signal, then how does affinity-based selection occur? We did observe a small fraction of GC B cells in which Syk is phosphorylated (fig. S5D) and SHP-1 relocated (Fig. 3D and fig. S7B) upon BCR ligation. We thus tested whether BCR signal transduction was dependent on the cell cycle. BCR ligation induced p-Syk only in GC cells within the G₂/M phase (Fig. 4, A and B). Commensurate with this, we found that dissociation of BCR and SHP-1 occurred in G₂; the amount of total SHP-1 was also substantially reduced in this phase but returned to normal levels at M phase (Fig. 4, C to F, and fig. S7, D and E).

The discovery that most highly proliferative GC B cells undergoing Ag-driven selection cannot execute BCR signaling was contrary to expectations. This posttranslational strategy for constraining signaling makes sense, however, as it could quickly be altered as cells progress through cycle or in response to other signals. These studies highlight a switch that gates early events in BCR signaling and that can be modified in GC B cells.

How, then, might affinity-based selection work? We suggest a model in which B cells integrate both T cell-derived and BCR signals in a cell-cycle-dependent manner. Studies suggest that individual BCRs are dedicated to either BCR signaling or Ag presentation, with only the former depending on canonical p-CD79 generation (24). GC BCRs, which do not generate p-CD79, may thus favor Ag presentation. Because BCR Ag capture is affinity dependent (25), more avid B cells would win out in a competition to gain T cell-dependent signals, for example, via CD40L. Such signals would likely promote survival over a longer time course (6) rather than instantaneously switch the outcome of BCR signaling by

reversing phosphatase activity, given the known kinetics and molecular connections between CD40 and NF- κ B signaling (26, 27).

Nonetheless, BCR signaling may be important at key times during GC B cell proliferative cycling. B cells must test their affinity with each round of somatic hypermutation (28). The testing of mutant phenotypes possibly occurs once per cell division, with the quality of BCR signaling determining the likelihood of completing mitosis and/or subsequent survival. Even in G₂, the threshold for effective Ag sensing appears elevated, thus favoring selection of higher-affinity B cells.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S8
References

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Restoring Voluntary Control of Locomotion after Paralyzing Spinal Cord Injury

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Half of human spinal cord injuries lead to chronic paralysis. Here, we introduce an electrochemical neuroprosthesis and a robotic postural interface designed to encourage supraspinally mediated movements in rats with paralyzing lesions. Despite the interruption of direct supraspinal pathways, the cortex regained the capacity to transform contextual information into task-specific commands to execute refined locomotion. This recovery relied on the extensive remodeling of cortical projections, including the formation of brainstem and intraspinal relays that restored qualitative control over electrochemically enabled lumbosacral circuitries. Automated treadmill-restricted training, which did not engage cortical neurons, failed to promote translesional plasticity and recovery. By encouraging active participation under functional states, our training paradigm triggered a cortex-dependent recovery that may improve function after similar injuries in humans.

Activity-based interventions exploiting proprioceptive information to enhance spinal motor output during training (1–3) promote plastic changes capable of restoring locomotion after severe though incomplete spinal cord injury (SCI) (3, 4). A recent case study suggests that, in combination with epidural electrical stimulation of lumbosacral segments, activity-based rehabilitation may also restore supraspinally mediated movements after motor complete paraplegia (5). We aimed to design a multisystem neuroprosthetic training program that took full

advantage of this concept. We hypothesized that, after the complete interruption of direct supraspinal input, a robotic postural interface encouraging the brain to actively use the paralyzed hindlimbs during electrochemically enabled motor states (6) would reestablish supraspinal control of locomotion by promoting extensive and ubiquitous remodeling of spared neuronal circuitries.

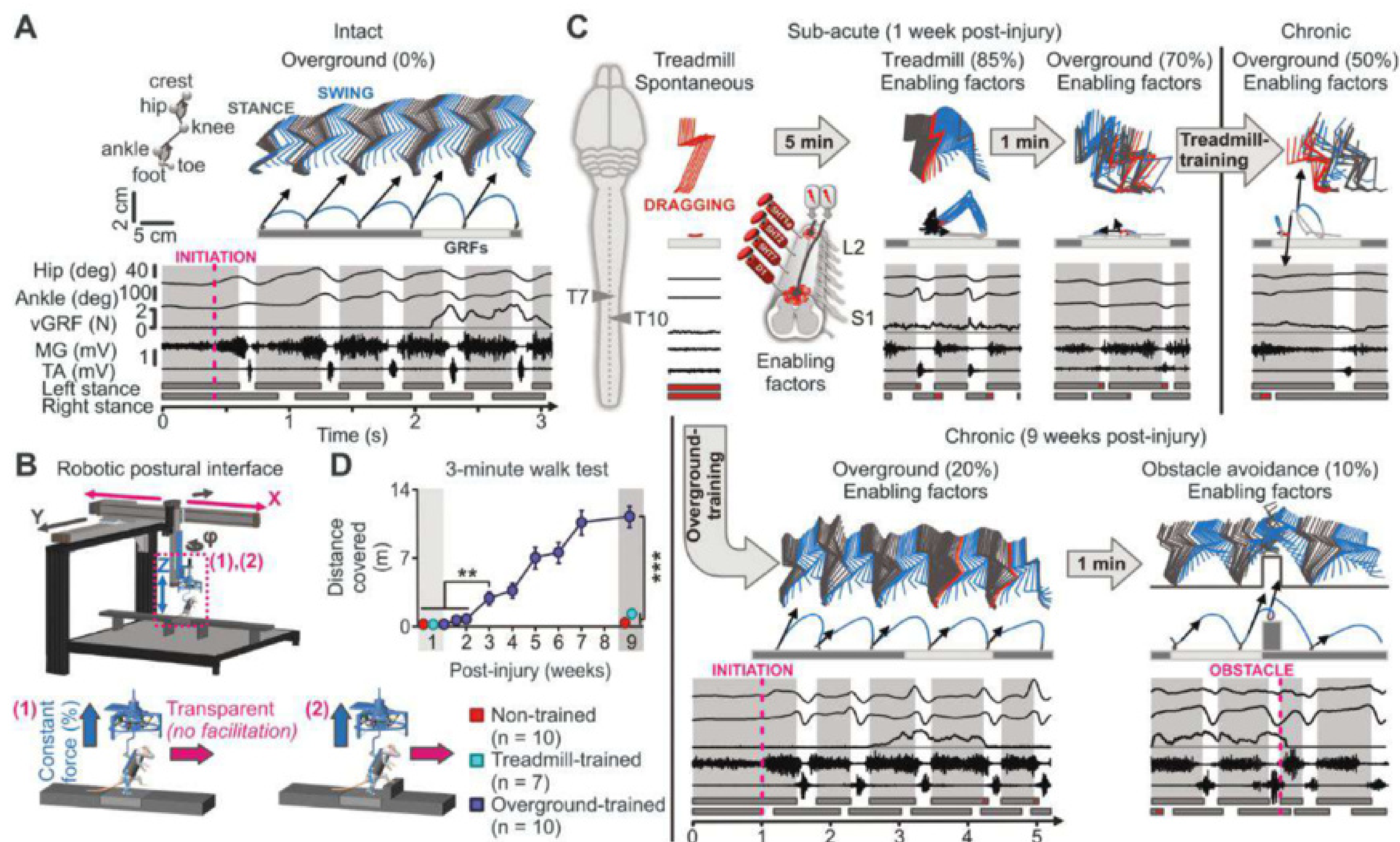
Adult rats received a left lateral over-hemisection at thoracic (T) vertebra T7 and a right lateral hemisection at T10. This SCI interrupts all direct supraspinal pathways (fig. S1, A to C), but leaves

an intervening gap of intact tissue. The lesion, however, led to a complete loss of hindlimb function, with no sign of recovery over 2 months post injury (fig. S1D). Likewise, humans with clinically complete SCI frequently show maintenance of connections through the lesion (7). Thus, this experimental lesion reproduces key anatomical and functional features of human SCIs, while providing well-controlled conditions to investigate the mechanisms underlying recovery (8).

To transform lumbosacral circuits from dormant to highly functional states (9), we applied tonic (40 Hz) epidural electrical stimulation over L2 and S1 spinal segments (6), and systemically administered a tailored cocktail of serotonin receptor agonists (5HT_{1A/7} and 5HT_{2A/C}) and dopamine (D₁) receptor agonists (10). By increasing the general level of spinal excitability, this electrochemical spinal neuroprosthesis enables sensory information to become a source of control for stepping (6, 9). This intervention promoted coordinated, although involuntary, bipedal stepping on a treadmill as early as 7 days post injury (Fig. 1C).

These stepping movements are elicited by the moving treadmill belt (6), which suggests that the

Fig. 1. Multisystem neuroprosthetic training restores voluntary locomotion after paralyzing SCI. (A) Left hindlimb kinematics, hindlimb end-point trajectory and velocity vector, vertical ground reaction forces (vGRF), as well as electromyographic (EMG) activity of medial gastrocnemius (MG) and TA muscles during bipedal locomotion in an intact rat. **(B)** Robotic postural interface providing vertical and lateral support, but no facilitation in the forward direction. **(C)** Representative left hindlimb stepping patterns recorded under the various experimental conditions 1 and 9 weeks post SCI. **(D)** Distance covered in 3 min during bipedal locomotion. %, Body weight support. ***P* < 0.01. ****P* < 0.001. Error bars, SEM.



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rats would not be capable of voluntarily initiating hindlimb locomotion overground. To verify the absence of supraspinal control, we applied the electrochemical neuroprosthesis and positioned the same rats bipedally in a robotic postural interface that provided adjustable vertical and lateral trunk support, but did not facilitate locomotion in any direction (Fig. 1B and fig. S2). All the rats ($n = 27$) failed to initiate hindlimb locomotion overground 7 days post injury ($P < 0.001$) (Fig. 1C).

We then designed a multisystem neuroprosthetic training program that encompassed two objectives. First, we aimed to improve the functionality of lumbosacral circuits through treadmill-based training enabled by the electrochemical neuroprosthesis (6). Second, we sought to promote the recovery of supraspinally mediated movements; we exploited the robotic postural interface not only to enable, but also to force, the rats to actively use their paralyzed hindlimbs in order to locomote bipedally toward a target.

Rats ($n = 10$) were trained daily for 30 min with a combination of both paradigms, starting 7 to 8 days post injury (fig. S3). The first, effortful voluntary steps emerged after 2 to 3 weeks of training ($P < 0.01$) (Fig. 1D). As voluntary movements recovered, we gradually increased the relative duration of overground training (fig. S3B). Five to 6 weeks post injury, all the rats (fig.

S4) were capable of initiating and sustaining full weight-bearing bipedal locomotion for extended periods of time, but only during electrochemically enabled motor states (Fig. 1, C and D, fig. S1D, and movie S1). Kinematic analyses (fig. S5) revealed that overground-trained rats deployed a similar control strategy as intact animals to produce locomotion (Fig. 1, A and C, and fig. S5). To measure recovery, we adapted the clinically standardized 6-min walk test (11) to bipedally stepping rats. Overground-trained animals with a paralyzing SCI covered distances as long as 21 m in 3 min (Fig. 1D).

We next tested whether treadmill-restricted step training under electrochemically enabled states would also promote the recovery of voluntary locomotion ($n = 7$ rats). This automated step training failed to reestablish overground locomotion despite repeated testing during 4 to 8 sessions 9 weeks post injury ($P < 0.001$) (Fig. 1, C and D, and movie S1). Moreover, treadmill-trained rats were not capable of sustaining robotically initiated locomotion overground (fig. S6).

To further enhance supraspinal contribution, we introduced stairs and obstacles; two conditions requiring voluntarily mediated gait tuning (12). After 2 to 3 additional weeks, overground-trained rats were capable of bipedally sprinting up stairs and avoiding obstacles (Fig. 1C, fig. S7, and movie S1). To accomplish these paradigms,

the animals displayed a range of task-specific adjustments of hindlimb movements (fig. S7).

Anatomical examinations highlighted an extensive remodeling of supraspinal and intraspinal projections in rats that regained voluntary locomotion. We first conducted retrograde tract tracing from lumbar (L) vertebrae L1/L2 locomotor centers (Fig. 2A). We found a significant increase ($P < 0.05$) (Fig. 2, B and C) in the number of labeled neurons in intermediate and ventral laminae of T8/T9 segments in both overground-trained and treadmill-trained rats compared with nontrained animals. Analysis of the activity-dependent marker, c-fos, after continuous overground locomotion confirmed that the labeled neurons were active during walking (Fig. 2F). The number of c-fos^{on} nuclei in the regions rich in neurons retrogradely labeled from L1/L2 locomotor centers was larger in overground-trained rats compared with all the other groups ($P < 0.01$) (Fig. 2, D and E). Thoracic neurons may thus play a pivotal role in restoring voluntary locomotion (8, 13, 14). To address this hypothesis, we ablated T8/T9 neurons by infusing the axon-sparing excitotoxin *N*-methyl-D-aspartic acid (NMDA) (8) (Fig. 2G and fig. S8). Infusion of NMDA abolished the regained voluntary locomotion ($P < 0.01$) (Fig. 2H and movie S2), despite uncompromised functionality of lumbosacral circuits (fig. S8). Likewise, overground-trained rats lost voluntary control of locomotion after the complete interruption of supraspinal input to T8/T9 neurons ($P < 0.01$) (Fig. 2, G and H).

We labeled projections from the left hindlimb motor cortex with injections of biotinylated dextran amine (BDA) (Fig. 3A). The bilateral interruption of the dorsal column at the T7 over-hemisection only spared a few (1 to 2%) (15) corticospinal tract (CST) axons in the right dorsolateral funiculus (fig. S9E). Consequently, nontrained rats showed scarce CST labeling in T8/T9 segments (Fig. 3, B and C, and fig. S9E). Treadmill-restricted training did not promote significant changes in the density of thoracic CST projections (Fig. 3, B and C, and fig. S9E). In contrast, we found a reconstitution of $45 \pm 7\%$ of prelesion bilateral fiber density in overground-trained rats (Fig. 3, B to D). These CST axons exclusively branched from the right dorsolateral funiculus (Fig. 3D), and they profusely innervated the right and, more unexpectedly, the left gray matter of T8/T9 segments (fig. S9F) (16). We detected multiple CST fibers extending from the gray matter at the T7 lesion site into the right dorsolateral funiculus (Fig. 3, E and F). These ectopic fibers, suggestive of regenerative sprouting (17), led to a near two-fold increase in the CST axon density of the T8/T9 dorsolateral funiculus ($P < 0.01$) (fig. S9G). Thoracic CST fibers bypassed the T7 over-hemisection through the right dorsolateral funiculus, branched into the gray matter, and recrossed the midline (Fig. 3E). These fibers developed large axonal structures with boutonlike swellings suggestive of sprouting in terminal arbors (fig. S9F).

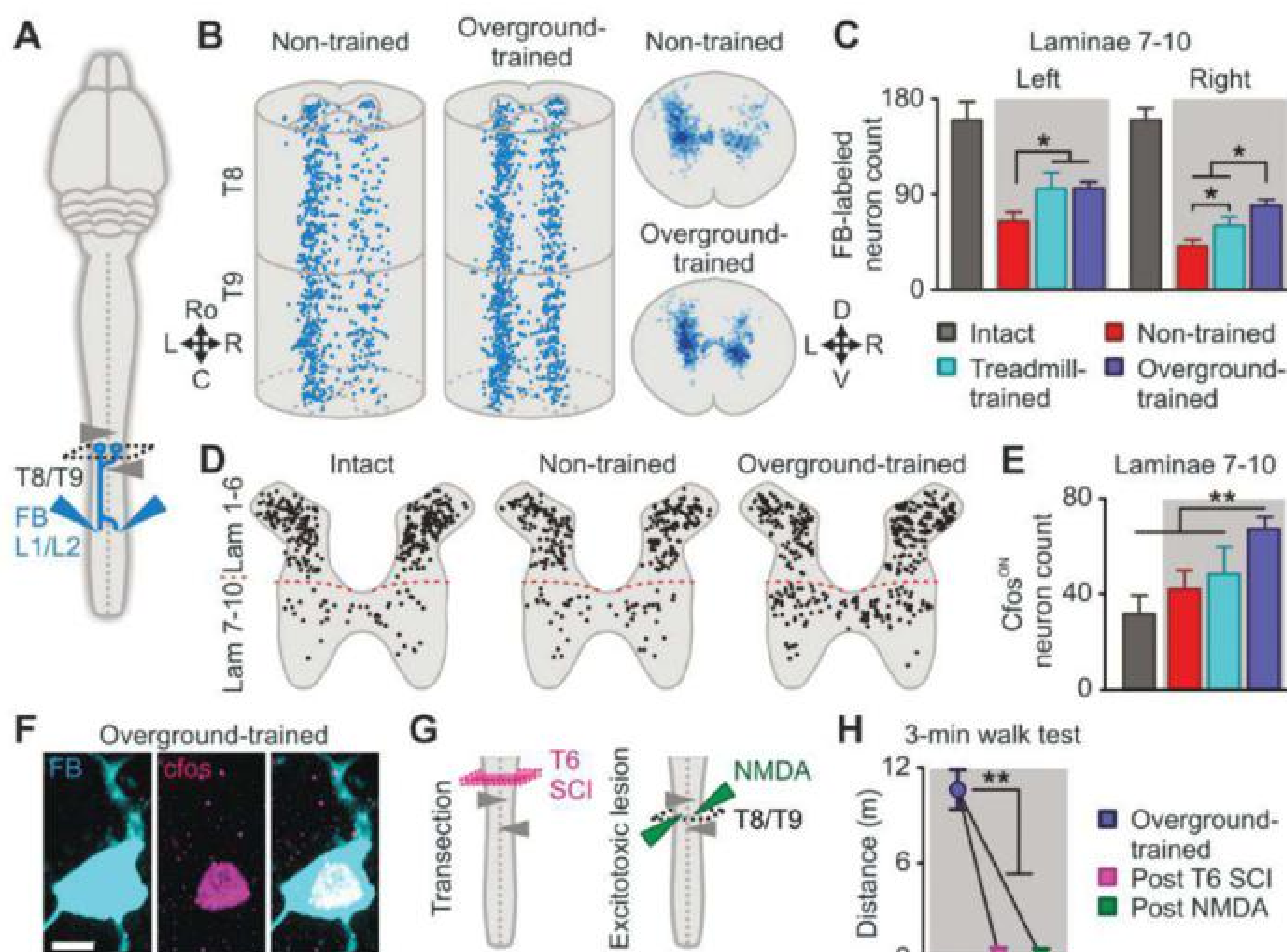


Fig. 2. Multisystem neuroprosthetic training promotes the formation of intraspinal detours that relay supraspinal information. (A) Diagram illustrating anatomical experiments. (B) Longitudinal and transverse views of three-dimensional (3D) reconstructions of Fast blue-labeled (FB) neurons between the lesions. L, left; R, right; Ro, rostral; C, caudal; D, dorsal; V, ventral. (C) Counts ($n = 6$ to 9 rats per group) of FB neurons in laminae 7 to 10 of T8/T9 segments after 45 min of continuous locomotion. (D) C-fos expression patterns in T8/T9 segments. (E) Counts ($n = 5$ to 7 rats per group) of c-fos^{on} neurons in laminae 7 to 10 after continuous locomotion. (F) Colocalization of FB and c-fos. Scale bar, 10 μ m. (G) Overground-trained rats received a complete T6 SCI ($n = 2$) or T8/T9 NMDA microinjections ($n = 3$). (H) Distance covered in 3 min before and after the lesions. * $P < 0.05$. ** $P < 0.01$. Error bars, SEM.

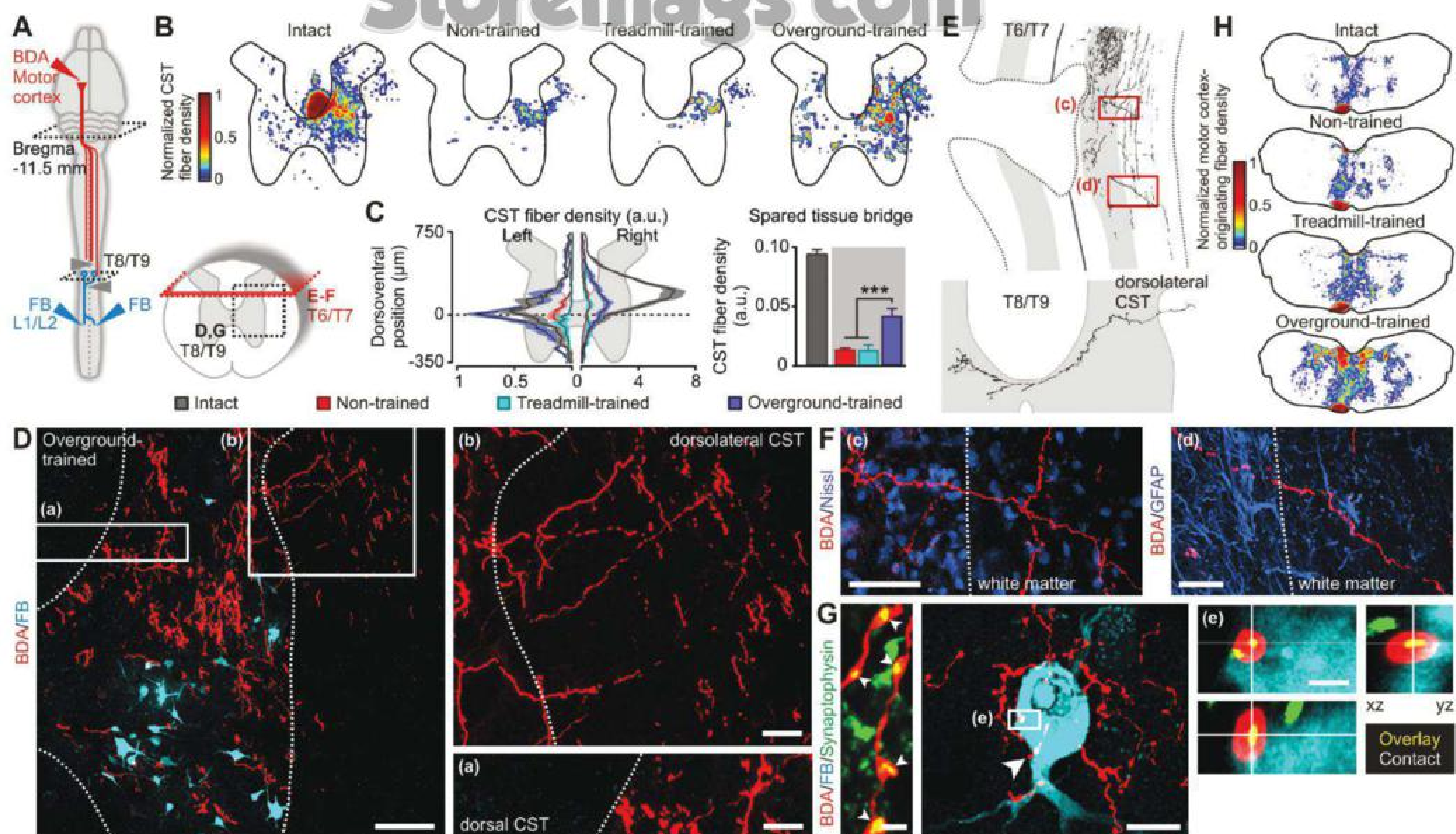


Fig. 3. Multisystem neuroprosthetic training promotes extensive remodeling of motor cortex projections. (A) Diagram illustrating anatomical experiments and analyzed regions. (B) Heat maps and (C) graphs ($n = 5$ rats per group) showing bilateral CST axon density in T8/T9 segments, a.u., arbitrary units. (D) Confocal overview of the right T8/T9 hemicord of an overground-trained rat. Scale bar, 100 μm ; insets [(a) and (b)], 30 μm . (E) 3D CST fiber reconstructions

along the longitudinal (top) and transverse (bottom) plane. (F) Confocal images of insets shown in (E). Scale bar, 50 μm . (G) Colocalization of CST fibers with synaptophysin (arrows), and close appositions (inset and arrow) with a FB neuron. Scale bar, 2 μm ; overview neuron, 10 μm . (H) Heat maps showing density of cortical projections in the brainstem (bregma -11.5 mm). *** $P < 0.001$. Error bars, SEM.

Confocal microscopy confirmed that thoracic CST fibers bore synaptic elements because they colocalized with synaptophysin (Fig. 3G). These fibers established contacts with relay neurons retrogradely labeled from L1/L2 locomotor centers (Fig. 3G).

Remodeling of motor cortex axonal projections was not restricted to the spared tissue bridge. Quantification of CST fibers at T4/T5, above the injury, revealed a significant bilateral increase of axon density in overground-trained compared with nontrained, treadmill-trained, and intact rats ($P < 0.01$) (fig. S9, A to D). We found a near fourfold increase in the density of cortical projections in various brainstem motor areas (Fig. 3H and fig. S10), including the left and right vestibular nuclei ($P < 0.01$), the entire reticular formation ($P < 0.001$), and parapyramidal regions ($P < 0.01$). These areas contain reticulospinal neurons and spinally projecting serotonergic neurons (fig. S10C) that both contribute to initiating and sustaining locomotion (18, 19). Descending 5HT fibers might thus reorganize with training. We found a nearly complete, lamina-specific restoration of T8/T9 serotonergic innervation in overground-trained rats, which contrasted with the depletion of 5HT fibers in nontrained and treadmill-trained animals ($P < 0.05$) (fig. S11).

Collectively, these analyses demonstrate that automated treadmill-restricted training failed to mediate anatomical changes in descending pathways, whereas active training under highly functional states promoted multilevel plasticity in cortex- and brainstem-derived axonal systems.

Contrary to primates, the rodent motor cortex is not essential to produce locomotion (20). Consequently, we sought to demonstrate that training-induced remodeling of motor cortex projections did contribute to controlling voluntary locomotion. First, we implanted stimulating epidural electrodes over the left motor cortex to verify that the reorganization of neuronal pathways reestablished connectivity across the lesion. Before the SCI, applying a train of low intensity (0.7 to 1.5 mA) electrical stimuli evoked large responses in the left tibialis anterior (TA) muscle (Fig. 4A). The SCI permanently abolished these responses in nontrained rats ($P < 0.001$) (Fig. 4A). In contrast, overground-trained rats regained responses below the lesion, averaging about 10% of their prelesion amplitude ($P < 0.001$) (Fig. 4B). These responses were delayed by 12 ± 3 ms ($P < 0.01$) (Fig. 4A), which suggests that a larger number of synaptic relays was necessary to convey the supraspinal volley to hindlimb motor pools. The amplitude of responses substantially increased during

electrochemically enabled motor states ($P < 0.01$) (Fig. 4, A and B), which indicated enhanced transmission of the supraspinal command (14). Second, we implanted a microwire array in the vicinity of CST neurons projecting to T8/T9 segments (Fig. 4C) and recorded neuronal modulations during voluntary locomotion in overground-trained rats ($n = 3$). We found a variety of neurons ($n = 17/24$ neurons) whose modulation patterns significantly ($P < 0.05$) (Fig. 4D) correlated with gait initiation, sustained locomotion, and corrective movements (fig. S12 and movie S3). A substantial number of motor cortex neurons (36%) exhibited a sharp increase in firing rate before any overt movement or locomotor-related muscle activity had occurred (Fig. 4E). Instead, the firing rate of motor cortex neurons significantly decreased during involuntary locomotion compared with quiet standing ($P < 0.05$) (fig. S13, A to C). Third, we inactivated the left motor cortex with a microinjection of the γ -aminobutyric acid (GABA) agonist muscimol (Fig. 4F). Muscimol immediately suppressed voluntary hindlimb locomotion ($P < 0.01$) (Fig. 4G and movie S3), despite uncompromised functionality of lumbosacral circuits (fig. S14).

Thus far, functional restoration after SCI has been interpreted as the need to promote long-distance regeneration of severed fibers to their

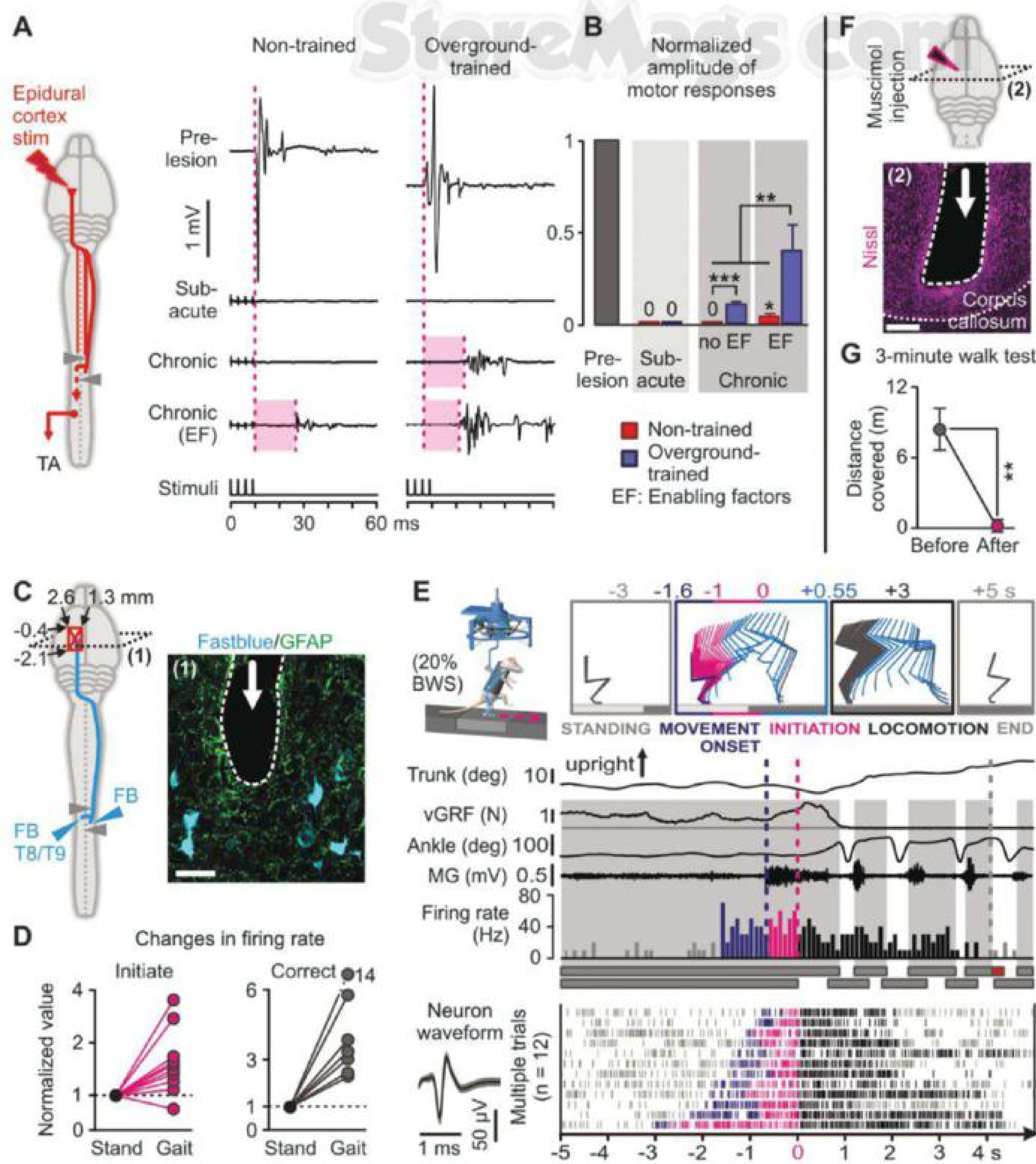


Fig. 4. Overground-trained rats regained cortical control of hindlimb locomotion. **(A)** Responses evoked by a train of epidural motor cortex stimulations in the left TA muscle in a nontrained and overground-trained rat. **(B)** Mean ($n = 5$ rats per group) amplitude of responses. **(C)** Diagram and GFAP staining to reveal glial fibrillary acidic protein (1) illustrating microwire array localization. Scale bar, 50 μm . **(D)** Change in firing rate for significantly modulated neurons during initiation and correction compared with standing. **(E)** Trunk vertical position, vGRFs, left ankle joint angle, EMG activity of left MG muscle, and modulation of a motor cortex neuron during one trial. Raster of the same neuron for multiple trials. Movement onset and initiation are defined as hip extension and foot clearance, respectively. The color-coding indicates the period during which firing rate significantly increased before these events. **(F)** Diagram and Nissl staining (2) showing catheter location for muscimol microinjection. Scale bar, 300 μm . **(G)** Distance covered in 3 min before and after muscimol injection. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. Error bars, SEM.

original targets (21, 22). Undoubtedly, neuroregeneration will be essential following near-complete SCI. However, a more immediate approach might capitalize on the remarkable capacity of spared neuronal systems to reorganize through use-dependent mechanisms (3, 5, 23). Here, we established training conditions that not only enabled but also forced the brain to construct a multiplicity of de novo brainstem and intraspinal relays to regain quantitative and qualitative access to electrochemically enabled lumbosacral circuitries. There is growing evidence that active training with appropriate sensory cues is markedly superior to passive, robot-guided rehabilitation to improve

stepping capacities in humans (3, 5, 23–26). Likewise, automated treadmill-restricted training, which did not engage cortical neurons, promoted sublesional plasticity, but failed to promote remodeling of descending pathways. Treadmill-trained rats did not regain supraspinally mediated locomotion. Instead, our new training paradigm encouraged active rat participation and triggered a cortex-dependent, activity-based process that restored voluntary control over sophisticated locomotor movements after a SCI that led to chronic paralysis. These results confirm the capacity of intraspinal circuits to bypass lesions (8, 13) and expand the therapeutic potential of detour circuits to the

restoration of function after paralyzing SCI. The ability of training under highly functional states to promote this extensive plasticity and recovery may lead to novel interventions capable of improving function in humans with a range of neuro-motor disorders (5, 27, 28).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6085/1182/DC1
Materials and Methods
Figs. S1 to S14
Table S1
References (29–32)
Movies S1 to S3

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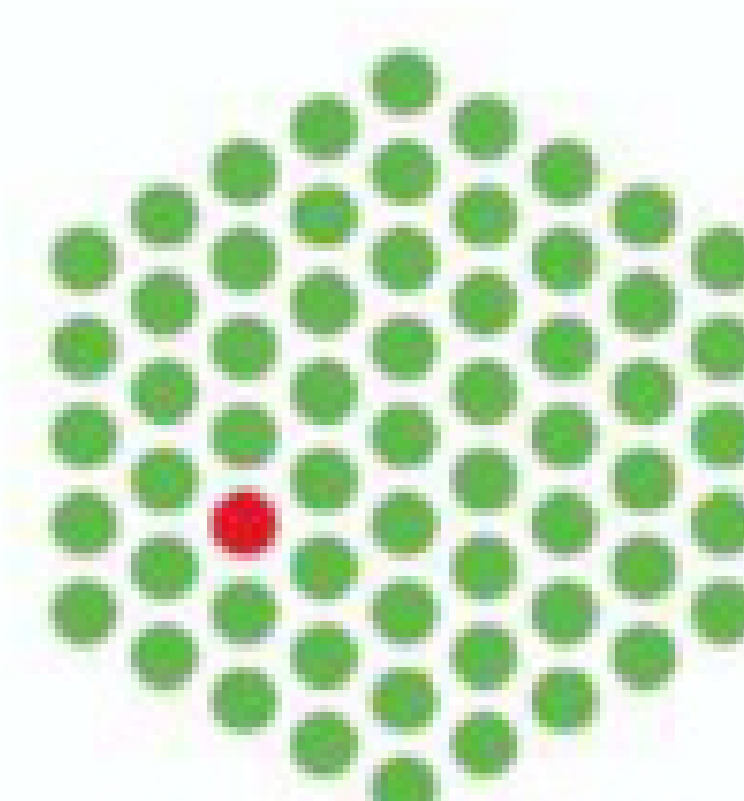
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**Director, Division of Clinical Innovation
National Center for Advancing Translational Sciences
National Institutes of Health
Department of Health and Human Services**

THE POSITION: The newly established National Center for Advancing Translational Sciences (NCATS) will catalyze the generation of innovative methods and technologies that will enhance the development, testing, and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions. As one of the National Institutes of Health Institutes/Centers, NCATS is not charged to focus on any one specific disease or human condition, but instead emphasizes the development of the tools and technologies to speed the process from basic discovery to better diagnostics and new treatments.

The Director, Division of Clinical Innovation (DCI), provides overall leadership and stewardship of NIH investments to bring innovation to the implementation of clinical research. A major component of the responsibilities of the DCI Division is oversight of the Clinical and Translational Science Awards (CTSA) program. The NCATS CTSA program is a major national investment in the translational sciences, providing academic homes at leading institutions across the country for the advancement and career development of investigators and supporting research resources needed by both local and national research communities to improve the quality and efficiency of all phases of translational research. This is a highly visible program and is expected to provide infrastructure for the full spectrum of translational research, leveraging both NCATS investments and investments from other NIH Institutes and Centers.

QUALIFICATIONS REQUIRED: Applicants must possess an M.D., or equivalent degree, as well as senior-level experience and leadership in the translation of basic science into clinical research. Candidates should be outstanding communicators with a scientific vision for the future needs of clinical research and demonstrated experience in running large, complex clinical research programs. Applicants should also demonstrate the ability to think strategically, work collaboratively and use a consultative approach to problem solving and decision making.

SALARY/BENEFITS/OTHER INFORMATION: Salary is commensurate with experience and a full package of Civil Service benefits is available, including: retirement, health and life insurance, long term care insurance, leave and savings plan (401K equivalent). The National Institutes of Health inspires public confidence in science by maintaining high ethical principles. In addition to the Federal government's code of ethics, we have our own agency specific standards - check them out at the NIH Ethics web site: <http://ethics.od.nih.gov/default.htm>. This position is subject to a background investigation.

HOW TO APPLY: Applications must include Curriculum Vitae, Bibliography, and a cover letter describing your vision for the future of translational and clinical research as well as how your qualifications match the needs of the position. The Search Committee will begin reviewing applications starting June 21, 2012 and will continue until a selection is made. Application packages should be sent to the **National Institutes of Health, National Center for Advancing Translational Sciences, ATTN: Terrie Squadere, 6701 Democracy Boulevard, Suite 900, Bethesda, Maryland 20892.**

For further information, please call (301) 451-1276. All information provided by candidates will remain confidential and will not be released outside the NCATS search process without a signed release from candidates.

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Researchers in Translational Ophthalmology

Heal the sick, advance the science, share the knowledge.

The Department of Ophthalmology at Mayo Clinic in Rochester is expanding its research division and is seeking scientists and clinicians with established translational programs focused in corneal disease, macular or retinal disease, or glaucoma. The successful applicants must demonstrate a track record of extramural funding, and be willing to collaborate with clinicians and scientists in different subspecialties of ophthalmology. Positions are equivalent to endowed professorships and include a competitive start-up package, and ongoing operating support. Credentials of successful applicants will include an MD or PhD (or equivalent) degree, national/international recognition in the field and a track record of NIH or equivalent peer-reviewed grant funding.

Recognized by *U.S. News and World Report* as one of America's Best Hospitals, Mayo Clinic is an excellent choice for the candidate who is seeking a career with a premier academic medical center, world-renowned for achievements and innovation. Mayo Clinic is a nonprofit organization with approximately 3,800 physicians and scientists across all locations, working in a collaborative environment focused on integrated patient care, research and education.

To apply and learn more about this position, Mayo Clinic and Rochester, MN, please visit www.mayoclinic.org/scientist-jobs/ and reference job posting number **8120BR**. Applications should include a letter of intent (addressed to Jay Erie, MD, Chair of Ophthalmology) and curriculum vitae and bibliography. Specific questions related to the posting should be directed to:

Jay C. Erie, MD
Chair and Professor of Ophthalmology
Mayo Clinic
200 First Street SW • Rochester, MN 55905
c/o schilbe.jennifer@mayo.edu

Mayo Foundation is an affirmative action and equal opportunity employer and educator. Post-offer/pre-employment drug screening is required.



Cardiovascular Physiology Faculty Position Department of Biomedical Sciences

The Department of Biomedical Sciences seeks to fill a tenure-track or tenured position at the Assistant to Associate Professor rank in Cardiovascular Physiology. Preference will be given to candidates with an established research program integrating cellular/molecular studies with whole-organism physiology. In addition, preference will be given to individuals whose interests complement other research groups in the department (neuroscience and/or reproductive physiology). The successful candidate is expected to maintain an independent, extramurally-funded research program and contribute to undergraduate, graduate, and/or professional veterinary medicine teaching.

Applicants must have a PhD, DVM, MD or equivalent degree and at least two years of postdoctoral training with demonstrated research productivity. In addition, applicants must provide evidence of competitiveness for independent national-level funding for their research program. A cover letter, curriculum vitae, statements of research and teaching interests, and names of three individuals willing to provide letters of reference, should be sent electronically to the chair of the search committee: Dr. Scott Earley, care of Karen Solomon, at Karen.Solomon@colostate.edu. Files of finalists will be available to all faculty members for review. Review of applications will begin **September 1, 2012** and continued to be accepted until a successful candidate is identified. www.cvmbs.colostate.edu/bms/

CSU is an EO/EE/AA Employer and conducts background checks on all final candidates.

ASSISTANT/ASSOCIATE PROFESSOR In Cardiovascular Research



Medical University of South Carolina

The Gazes Cardiac Research Institute in the Cardiology Division of the Department of Medicine at the Medical University of South Carolina is seeking applicants for a tenured-track faculty position of Assistant/Associate Professor. The successful candidate will be expected to establish an externally funded research program focused on the mechanisms mediating complex cardiac arrhythmias and sudden death and will be expected to integrate their research program into the highly collaborative and supportive existing research program. Candidates must have a Ph.D. or M.D. in a related field and rigorous postdoctoral experience leading to publications in top tier journals. The Gazes Cardiac Research Institute represents a cohesive group of 6 nationally funded investigators whose labs are well equipped to perform state of the art basic and translational cardiovascular research. Areas of current expertise include factors affecting adult cardiac hypertrophic growth, gene regulation, translational and post-translational modification events, cardiac fibrosis, remodeling following MI and pressure overload, diastolic dysfunction, and cardiac conduction system biology and remodeling.

Interested individuals should submit a letter of interest, a curriculum vitae, a detailed statement of research plans, 3 representative publications and the names and contact information of three references via the MUSC employment website at <https://www.jobs.musc.edu> requisition ID **048268**.

Inquiries regarding this position can be sent to: Donald Menick, Ph.D., Director, Gazes Cardiac Research Institute via menickd@muscd.edu.

Review of Applications will begin immediately and will continue until the position is filled.

*MUSC is an Equal Opportunity Employer,
promoting workplace diversity.*



School of Medicine Department of Medicine Endocrinology Assistant or Associate Professor Tenure/Tenure-track

University of California Irvine Diabetes Center

The Diabetes Center and the Endocrinology Division, Department of Medicine at UC Irvine are accepting applications from qualified physician-scientists for a tenure track/tenured faculty position as Assistant/Associate Professor in the In-Line series. The successful candidate should have a strong background and training in basic and/or translational research related to diabetes and metabolic disorders.

This position is integrated with PRIME-LC, a UCI School of Medicine program focusing on health issues of the growing Latino population. This faculty member will be given protected time to establish an extramurally funded, independent research program in the areas of diabetes, obesity, or metabolic syndrome. The candidate will participate in clinical and educational activities of the Endocrinology division and should have completed an endocrinology fellowship training program and be board certified/eligible in Internal Medicine and Endocrinology.

The successful candidate will be offered a highly competitive start-up package, laboratory space, and fringe benefits. For further information regarding this position, please contact: **Ping H. Wang, MD, Chair, Search Committee, UCI Diabetes Center, phwang@uci.edu.**

Applicants should complete an online application profile and upload the following application materials electronically to be considered for the position: Cover letter, curriculum vitae, statement of research and teaching interests, representative publications, and identification of five or more references. Please log onto UC Irvine's RECRUIT located at <https://recruit.ap.uci.edu/apply>.

The University of California, Irvine School of Medicine is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual-career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF Advance Award for gender equity.

Research Positions at Vale Technological Institute

Vale Technological Institute (ITV) is a recently established Brazilian research center focused on science, technology and innovation with particular emphasis on the exploration of natural resources, mining, habitat conservation and sustainable development. The research facilities of ITV are located in Belém, Para and in Ouro Preto, Minas Gerais. We seek to employ scientists with strong track records in research, teaching, ability to work in multidisciplinary teams and in attracting external funding. Applicants should have at least 5 years postdoctoral research experience and ability to conduct independent research.

Positions are now open for:

- * **Hydrogeologist** - experience in monitoring physical-chemical properties of water and in the evaluation and use of surface and underground water resources.
- * **Environmental Geochemist** - emphasis on water and soil geochemistry, preferably with experience in the Amazon and a solid track record in the assessment of environmental impact of mining activities.
- * **Geographer or Environmental Engineer** - research experience in the use and exploitation of water resources, including management of environmental and social aspects and their valuation.
- * **Electron Microscopist** - a geologist, material scientist, chemist, or metallurgical engineer with experience in scanning electron microscopy (obtaining and interpreting images and analytical data).

- * **Regional Economist (focus on Spatial Dimension of Economics)** - strong competencies in regional economics and spatial econometrics or spatial analysis, and interest in empirical economic research within Regional Economics.
- * **Ecological or Environmental Economist** - competence in ecological economics and quantitative methods for environmental economics.
- * **Development Economist** - strong competencies in economics of development, on related quantitative methods and questions of inequality.
- * **Biodiversity Group:** Two positions are currently open.
 - Systems biologist / bioinformatician with manifest expertise in the analysis of microbial metagenoms
 - Molecular biologist / microbiologist with experience in isolating DNA from the soil metagenome and the production and screening of clone libraries by functional and sequence-based approaches.

Closing date of applications: June, 22

To apply, please submit your CV and a covering letter confirming your current salary and any other important information to: itvjob@vale.com

www.vale.com

VALE TECHNOLOGICAL INSTITUTE



ISTFELLOW

IST AUSTRIA IS LOOKING FOR POSTDOCTORAL FELLOWS

IST Austria (Institute of Science and Technology Austria) invites applications for postdoctoral fellows in all fields of the natural and mathematical sciences and related disciplines.

The Institute, which is on the outskirts of Vienna, was established by the Austrian government with a focus on basic research. IST Austria has English as its working language, and its Graduate School awards PhD degrees. It has an international mix of scientists chosen solely on the basis of their individual excellence and potential contribution to research.

The Institute has set up a program for exceptional postdoctoral researchers partially funded by the European Union. Appointments will be for 2–4 years. Applications will be accepted at any time, but fellows will be selected twice a year in October and April.

The deadlines for each selection are the 15th of September and March, respectively. Applicants must have the support of one or more Professors or Assistant Professors of IST Austria who will host them in their research group.

The Institute offers an internationally competitive salary, full social security coverage, and additional benefits.

For further information about the program and the online application process, please refer to the ISTFELLOW website: <http://ist.ac.at/istfellow>

IST Austria values diversity and is committed to equality. Female researchers are encouraged to apply.





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Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

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To learn more, visit aaas.org/plusyou/sciencecareers





EMBL-EBI



EMBL-EBI and Wellcome Trust Sanger Institute share the Wellcome Trust Genome Campus near Cambridge. This proximity fosters close collaborations and contributes to an international and vibrant campus environment.

ESPOD fellowships

Call for applications 2012

The EMBL-EBI – Sanger Postdoctoral (ESPOD) Programme builds on the strong collaborative relationship between the two institutes, offering projects which combine experimental (wet lab) and computational approaches.

Propose your own project or select from pre-defined projects:

- Systematic characterisation of Sanger human iPS cells
- Modelling the genetic-epigenetic regulatory pathways in hematopoietic cells
- Genes, pathways, modules and organs that regulate bone mass
- Comparative genomic insights into parasite genome function
- Adaptive evolution of olfactory systems in mammals

For further information on the ESPOD fellowships and how to apply please visit www.ebi.ac.uk/training/postdoc/ESPOD

Closing date: 15 August 2012

www.embl.org/jobs



The EGL Foundation invites you to apply to the

Gruss Lipper Post-Doctoral Fellowship Program

Eligibility

- Israeli citizenship
- Candidates must have completed PhD and/or MD/PhD degrees in the Biomedical Sciences at an accredited Israeli University/Medical School or be in their final year of study
- Candidates must have been awarded a postdoctoral position in the U.S. host research institution.

Details regarding the fellowship are available at www.eglc.org

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POSITIONS OPEN



FACULTY POSITION

Department of Microbiology, Immunology & Parasitology
Louisiana State University Health New Orleans
School of Medicine New Orleans

The Department of Microbiology, Immunology & Parasitology in the School of Medicine at Louisiana State University Health New Orleans, LA (LSU Health-NO) invites candidates with established research programs to apply for a faculty position on the tenure-track as **ASSOCIATE** or **FULL PROFESSOR**. Candidates should have a strong record of research accomplishment, lead an active, nationally funded research program, and have a commitment to developing a collaborative translational research program involving multiple investigators. Expertise in all areas of microbiology and immunology will be considered, but special consideration will be given to those complementing existing core departmental strengths in HIV and HIV-related infections including pneumocystis and tuberculosis, herpesviruses/host interactions, sexually transmitted diseases including Chlamydia infection, molecular mycology, and the microbiome. LSU Health-NO School of Medicine offers a highly interactive and collegial environment, with a strong history of collaborative research programs and state-of-the-art infrastructure, including core laboratories in genomics, proteomics, imaging, and flow cytometry. Excellent opportunities exist for interaction with clinical departments, and with Research Centers of Excellence in Vaccine Development, Cancer, Alcohol and Drug Abuse, Cardiovascular Biology, and Neuroscience. Anticipated duties and responsibilities will include sustaining an exceptional research program, mentoring of graduate students and postdoctoral fellows, and participating in departmental and school graduate and undergraduate teaching programs. The institution offers competitive startup packages and salaries. Applicants should send their curriculum vitae that includes previous and current research funding, teaching experience, a statement of research plans, and the names of at least three references to **e-mail: keigen@lsuhsc.edu**. *LSU Health-NO is an Equal Opportunity/Affirmative Action Employer. Reference #82020.*

UNDERGRADUATE ADVISER and INSTRUCTOR

University of Oregon
Department of Biology

The Department of Biology at the University of Oregon (UO) seeks a dynamic and energetic professional to teach undergraduate biology courses, participate in graduate student recruitment, and join the Biology Undergraduate Advising Center to provide undergraduate student advising, evaluative services, and outreach. Successful candidates will be committed to supporting the university's emphasis on academic excellence in a culturally diverse and academically challenging learning environment. Master's degree required; Ph.D. in biology or closely related field preferred. This is a nine-month, fixed-term, up to full-time appointment starting September 16, 2012 with annual renewal anticipated pending satisfactory performance, sufficient funding, and departmental need. Salary: \$37,500–\$45,000 (nine-month full-time annual rate; salary will be prorated during any terms when employment is part time). See ad on the UO website: <http://jobs.uoregon.edu/unclassified.php?id=3882> for full position description and requirements. Applications accepted online at website: <https://academicjobsonline.org/ajo/jobs/1524>. Applicants should submit a cover letter, curriculum vitae, a statement of purpose, and three letters of recommendation. The deadline to upload application materials is June 22, 2012. Position is subject to criminal background check.

Women and minorities are encouraged to apply. The University of Oregon is an Equal Opportunity/Affirmative Action Institution committed to cultural diversity and compliance with the Americans with Disabilities Act, and supportive of the needs of dual career couples. We invite applications from qualified candidates who share our commitment to diversity.

POSITIONS OPEN



MENTAL HEALTH/ BEHAVIORAL SCIENTIST Assistant/Associate Professor (Tenure track)

The Center for Molecular and Behavioral Neuroscience (CMBN) at Meharry Medical College invites applications for a tenure-track position at the Assistant or Associate Professor level. The successful candidate will be expected to develop an independent research, obtain extramural funding, and be involved in Ph.D./residency-level training program that enhances translational, clinical and human behavioral/mental health research in CMBN, the Center for Women's Health Research, and the Center for AIDS Health Disparities Research at Meharry Medical College.

CMBN's mission is to conduct basic, translational, clinical, social and behavioral research to generate new knowledge that will contribute to the nation's effort to reduce health disparities in the areas of neurological disease, mental health as well as drug abuse and addiction, including alcoholism, while contributing significantly to the production of the next generation of behavioral and neuroscientists. Faculty members in the center are engaged in bench-to-beside-to-community research on mechanisms of addictive disorders, Alzheimer's disease, mood disorders, neuro-degeneration, neuron-protection strategies, neuro-toxicology, Parkinson's disease, risk-taking behavior, and sleep disorders.

Applicants must have a doctoral degree (M.D. and/or Ph.D.) in a field relevant to behavioral science/mental health and appropriate postdoctoral experience. The academic appointment will be in the department that is most consistent with the educational background and research interests of the candidate. CMBN's investigators currently hold academic appointments in the departments of Neuroscience and Pharmacology, Physiology, and Psychiatry and Behavioral Sciences.

Located in Nashville, Tennessee, Meharry Medical College is the largest private, historically black institution exclusively dedicated to educating biomedical scientists and healthcare professionals in the United States. The institution is composed of the Schools of Dentistry, Graduate Studies and Medicine.

The screening of applications is ongoing and the position will remain open until a suitable candidate is identified. Interested individuals should submit a letter of interest summarizing their qualifications and future research plans, along with curriculum vitae and contact information for three references to:

Uma Rao, M.D.

Director, Center for Molecular and Behavioral Neuroscience
Professor, Psychiatry and Behavioral Sciences
Meharry Medical College
1005 D. B. Todd Blvd
Nashville, TN. 37208
E-mail: urao@mmc.edu

Meharry Medical College is an Equal Opportunity Employer.

POSTDOCTORAL RESEARCHER POSITION Davis Heart & Lung Research Institute The Ohio State University

A Postdoctoral Researcher position is available in the laboratory of **Dr. Jay L. Zweier** at the Davis Heart and Lung Research Institute, Ohio State University (OSU), Columbus, Ohio, USA. The candidate should have strong cardiovascular physiology and molecular background in rodent models of myocardial ischemia-reperfusion. The overall goal of the laboratory is to better understand the mechanisms of postischemic injury, cardioprotection, and myocardial regeneration in relation to free radical and nitric oxide formation. A M.D. or Ph.D. with at least two years experience in rodent myocardial infarction models and basic biochemical and molecular techniques is required. Salary commensurate with experience. Please send the following materials to **e-mail: jay.zweier@osumc.edu**: a cover letter, curriculum vitae, related peer-reviewed articles, and contact information of three references. *OSU is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

FACULTY POSITION

The Department of Molecular and Cell Biology at the Boston University Henry M. Goldman School of Dental Medicine occupies a completely renovated floor adjacent to basic science departments of the Medical School. We have an opening at the **ASSISTANT, ASSOCIATE, or FULL PROFESSOR** level. We seek individuals with an outstanding publication record and an ongoing NIH R01 or K99/R00-funded research program as principal investigators. We seek qualified candidates with research interests in cell and developmental biology, molecular genetics, biochemistry, immunology, or microbiology. Interest in craniofacial and oral biology is encouraged but not necessary. Excellent laboratory facilities and startup funds are available as well as joint appointments with appropriate departments at the School of Medicine and participation in the Bioinformatics Program at the School of Engineering. Qualified applicants should submit curriculum vitae, a brief summary of research accomplishments, a description of plans for future work, and names of three to five references, by July 31, 2012. Electronically send all materials to: **Dr. D.E. Levin**, Department Chair, at **e-mail: mcbdept@bu.edu**.

Please visit website: <http://dentalschool.bu.edu/research/molecular/index.html>. *Boston University is an Affirmative Action/Equal Opportunity Employer.*

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WEBINAR

Optical Sectioning using Light Sheet Microscopy

In Vivo Imaging with
Astounding Resolution

June 14, 2012

12 noon ET, 9 a.m. PT, 4 p.m. GMT, 5 p.m. UK

Light sheet microscopy is an extremely powerful alternative to established fluorescence imaging techniques, especially when it comes to 3-D imaging deep within tissue or within whole live organisms. By selectively illuminating the observed optical section with a thin sheet of light, photo bleaching is reduced to a minimum, making light sheet microscopy ideal for nondestructive imaging of fragile samples over extended periods of time. Since such orthogonal arrangements provide true optical sectioning capability, appropriately prepared millimeter-sized samples can be imaged extremely fast and with remarkable resolution and penetration depth. Our panel of experts will introduce the audience to this novel and powerful technique, providing real-world examples of its application in a variety of research settings.

During the webinar, our speakers will:

- Give a brief summary of the technique, its advantages, and its challenges
- Present the advances in their research made possible through the use of light sheet microscopy
- Answer questions submitted by you!

Participating Speakers

Ernst H. K. Stelzer, Ph.D.

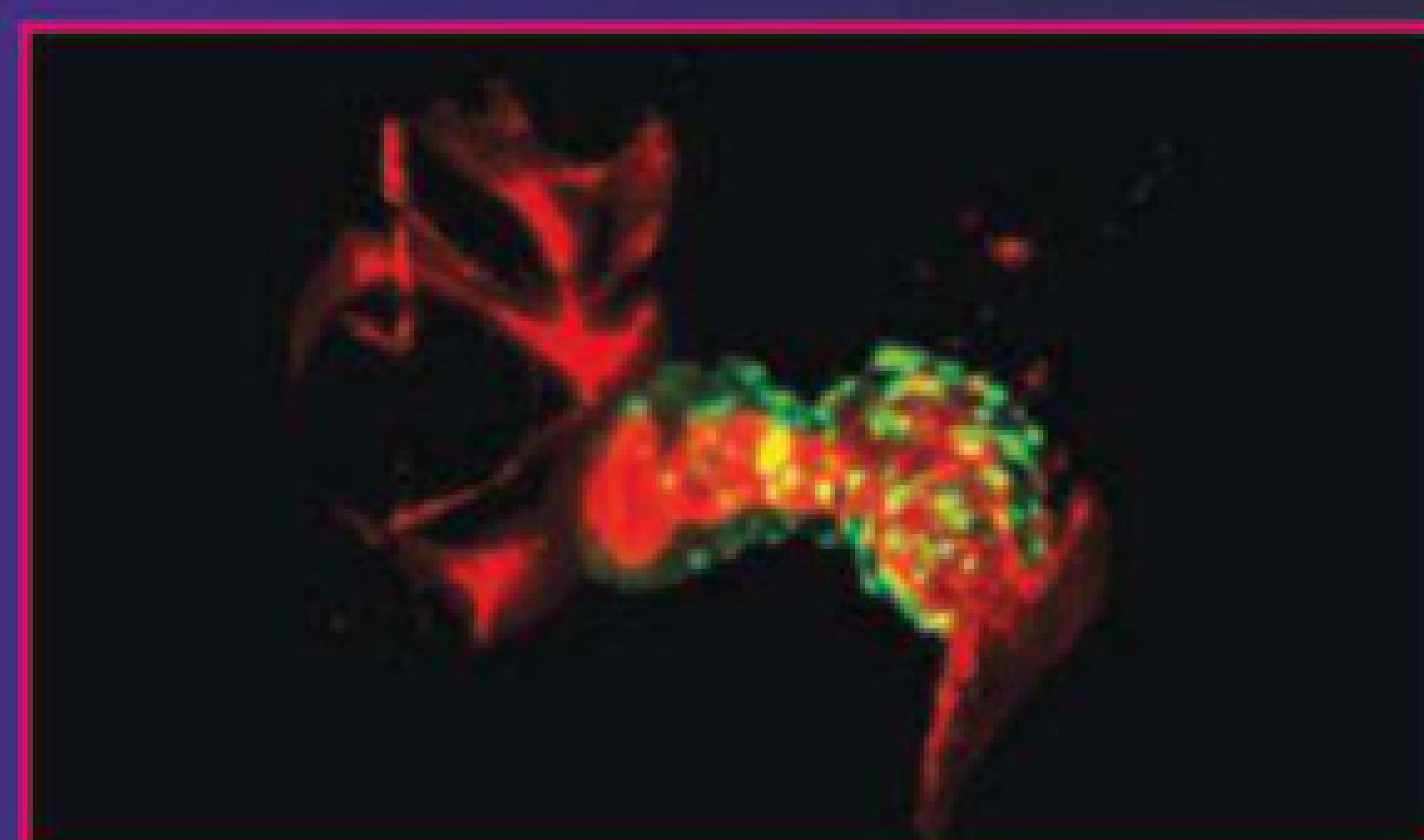
Goethe University
Frankfurt, Germany

Pavel Tomancak, Ph.D.

Max Planck Institute of Molecular
Cell Biology and Genetics
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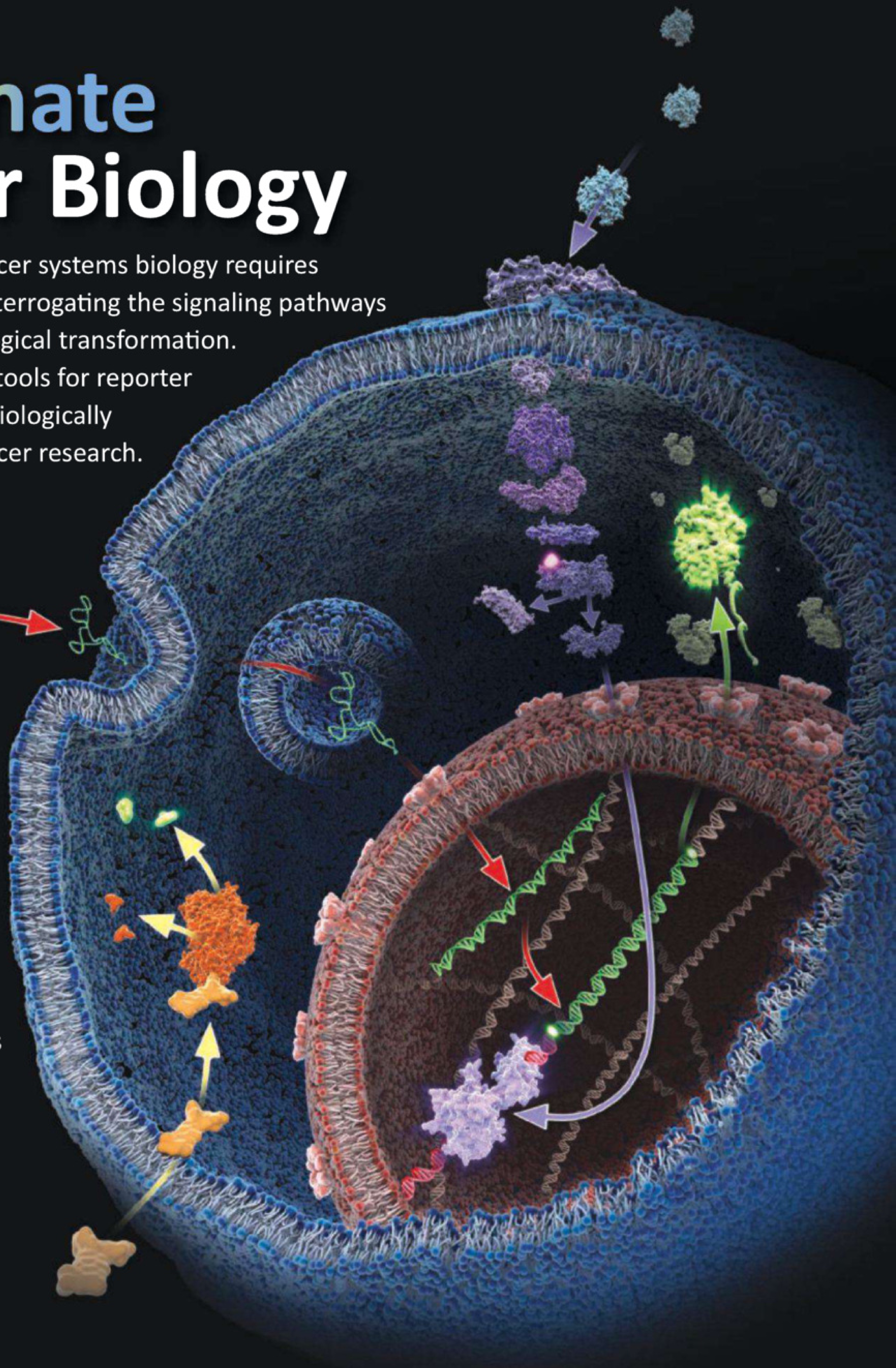
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